A THESIS ENTITLED

"MODERN NMR METHODS IN THE STRUCTURAL ELUCIDATION OF NATURAL PRODUCTS"

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CONTENTS

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MODERN NMR METHODS IN THE STRUCTURAL ELUCIDATION OF NATURAL PRODUCTS

Tahid

SUMMARY

The thesis consists of a General Introduction, dealing briefly with the biogenesis of terpenoids and five chapters. Chapter I is concerned with a review of the tetranortriterpenoids, a group of modified triterpenoids from the Meliaceae and Rutaceae families. This is followed by discussion of the results of investigations into the tetranortriterpenoid constituents of the wood of *Vavaea amicorum* in Chapter II. The isolation of the protolimonoids bourjotinolone A and a new epoxide related to bourjotinolone C is described. Their structures were elucidated mainly using ^{1}H and ^{13}C NMR spectroscopy. Chapter III describes the investigation of some sesquiterpenes from *Turraea brownii*. Humulene, a mixture of humulene epoxide and caryophyllene epoxide and three other unknown sesquiterpenoids belonging to the guaiane, bisabolene and germacrane series, were obtained. Guaiaol, bisabolone and hydroxy germacrene structures are proposed for these sesquiterpenoids. Computer assisted structure elucidation based on correlations from HMBC and HMQC experiments was used to resolve the problem of the mixture of humulene epoxide and caryophyllene epoxide. The investigation of cedrelone and others limonoids in the species *Toona australis* is discussed in Chapter IV. In the final Chapter V, the isolation of an interesting compound from the Indonesian traditional medicinal plant, *Hemigraphis alternata*, is described. Unfortunately this compound is still a mixture. The spectroscopic properties of the mixture are presented. The components of the mixture may well be diterpenoid in character.

GENERAL INTRODUCTION

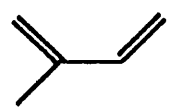
INTRODUCTION

Natural product chemistry has undergone great changes during the last twenty years. Dramatic progress in nuclear magnetic resonance (NMR) techniques has greatly facilitated structural and biosynthetic studies of natural compounds. Important progress has also been made in isolation, separation, and purification methods. It is now possible to isolate microgram quantities of a compound of interest and elucidate its structure[1].

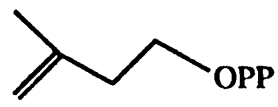
Most natural products have been obtained from plants or microorganisms and can be divided into two broad groups, primary and secondary metabolites. The fundamental molecules of living matter such as carbohydrates, proteins, fats, lipids, amino acids, and nucleic acids are regarded as the primary metabolites. Although not essential for the existence of the individual organism, secondary metabolites such as terpenoids, alkaloids and pigments often play a key role in the species.[2,3] The terpenoids form one of the most widespread and chemically interesting groups of natural products. Research in this area, including the isolation of new compounds, structural elucidation, chemical synthesis and biosynthetic studies, continues unabated.[4,5,6]

A number of terpenoids possess physiological importance for the growth and reproductive functions of higher and lower plants, e.g.; cytokinins, gibberellins, abscisic acid, and sterols.[7] Member of the class, particularly lower terpenoids, are commercially valuable as ingredients of flavours, soap, perfumes, drugs and pigments. Several sesquiterpenes exhibit interesting antibiotic bioactivity[8], while others display antifungal activity.[9] Many highly oxygenated sesquiterpenes show significant antitumor cytotoxic activity.[10] In the field of agriculture, some terpenes and their derivatives such as pyrethrin, derivatives of chrysanthemic acid, and the bisabolene sesquiterpene juvabione play a major role in the pesticide industry.[11]

The idea that the structural diversity of terpenoids can be unified for the whole class on the basis of their biogenesis has attracted much attention for many years. Although some details of the biosynthetic pathways of terpenoids are still being elucidated it was in 1887 Wallach recognized that terpene structures were constructed from isoprene units (1) linked "head-to-tail".



(1)



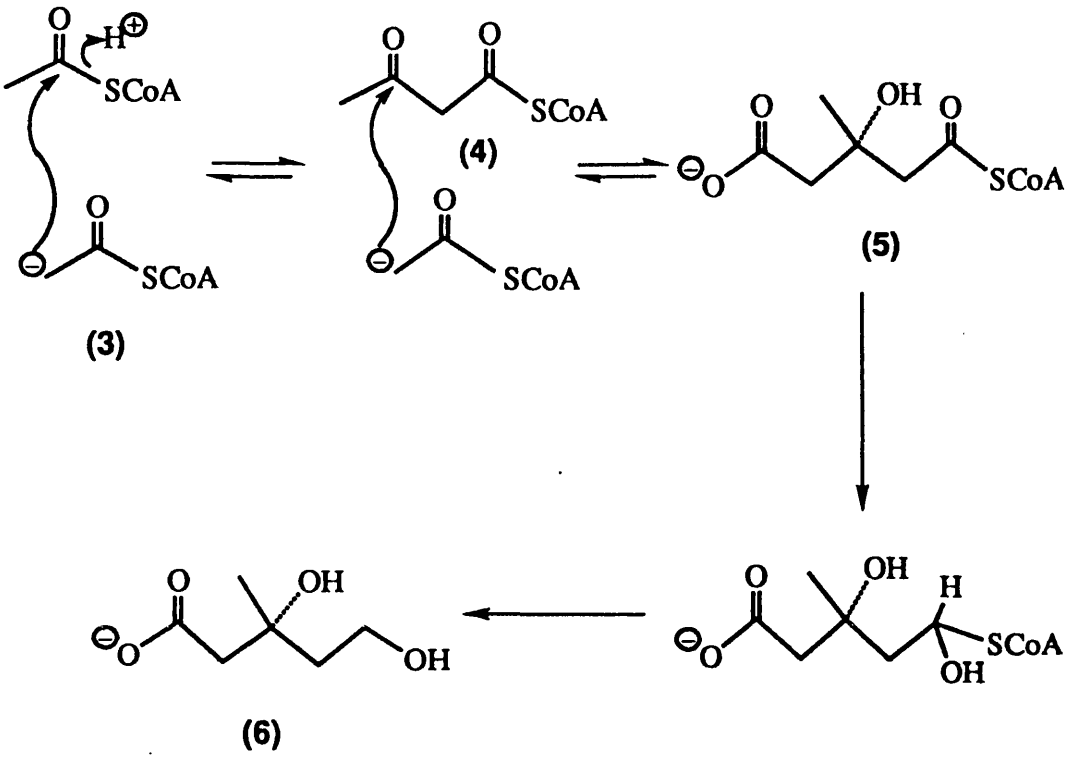
(2)

This "Isoprene Rule" was later modified by Ruzicka[12] who proposed a "Biogenetic Isoprene Rule" to account for the terpenoids whose skeleton cannot be constructed from isoprene units. The "Biogenetic Isoprene Rule proposed that all terpenoids are synthesized from a common precursor ("active isoprene") which turned out to be mevalonic acid.

TERPENOID BIOSYNTHESIS

Following much speculation about the mode by which isoprenoid units are constructed and combined biosynthetically to produce terpenoids it is now clear that they are derived from mevalonic acid the immediate precursor of the "isoprene unit" isopentenyl pyrophosphate [IPP] (2).[7,13]

The tranformation of (3*R*)-mevalonic acid (6) [MVA] into cholesterol in rat liver was demonstrated by Folkers and co-workers in 1956 and the result suggested that MVA is the key precursor of isopentenyl pyrophosphate (IPP)[14]. Subsequent studies proved that acetic acid, in the form of acetyl coenzyme A, is the basic precursor of terpenoid biosynthesis. The formation of IPP from acetyl-CoA is shown in Scheme 1 as follows;



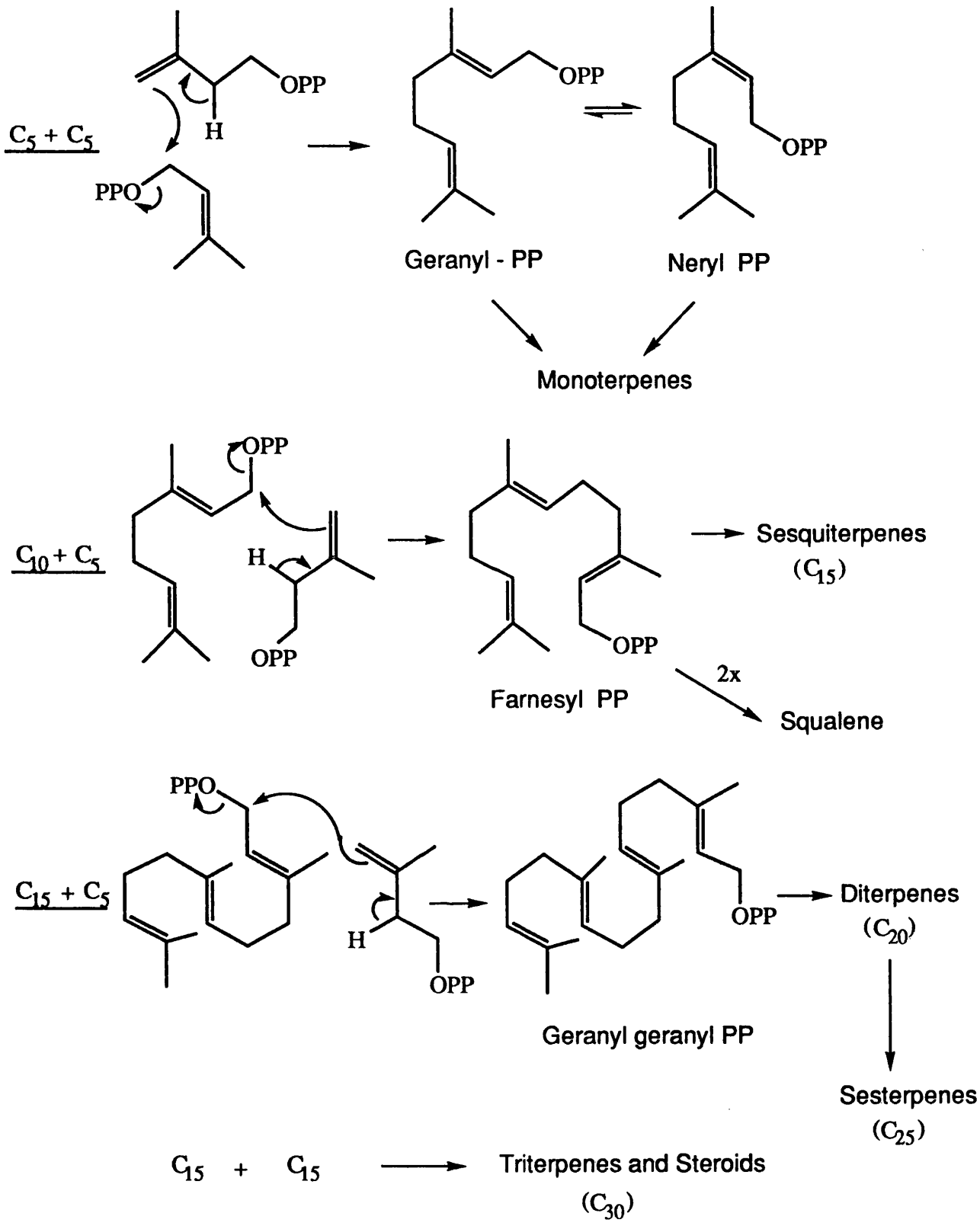
Scheme 1

Six Carbon Intermediates.

The route for the conversion of acetate to mevalonic acid starts with the combination of two moles of acetyl coenzyme A (**3**) (see Scheme 1) to produce acetoacetyl coenzyme A (**4**). Enzymatic condensation of (**4**) with another mole of acetyl coenzyme A affords (3*S*)-3-hydroxymethylglutaryl coenzyme A (**5**) with release of coenzyme A. Enzymic reduction, by two hydrogen transfers from reduced NADPH of (3*S*)-3-hydroxymethylglutaryl-CoA leads to MVA.[7] Only the*R*-form of MVA is utilised in terpenoid biosynthesis.[13]

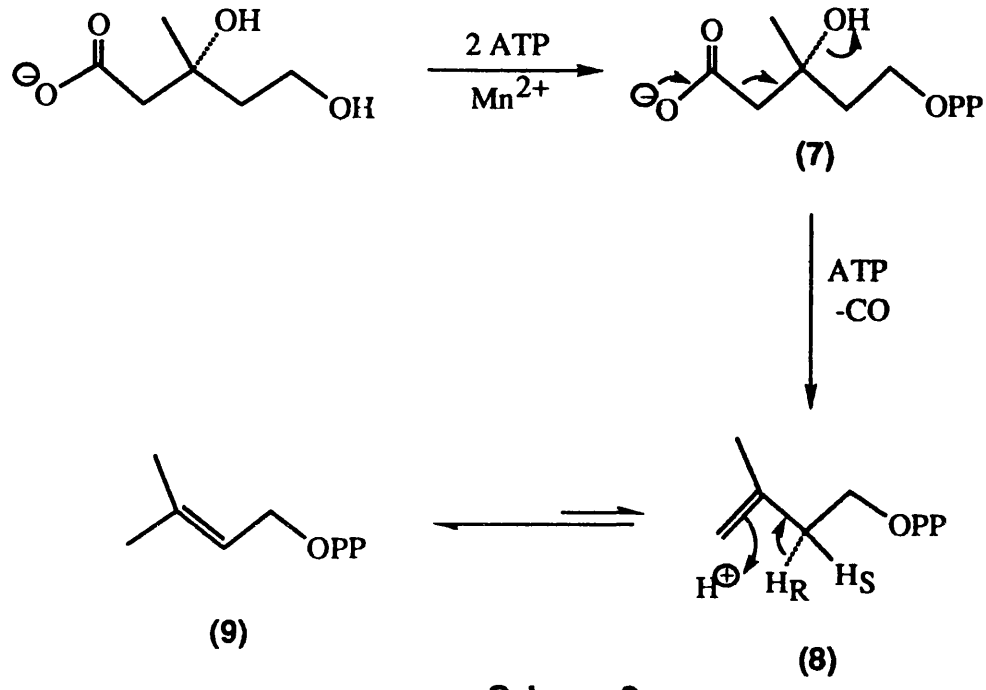
Five Carbon Intermediates.

The conversion of MVA (**6**) to its pyrophosphate involve phosphorylation by ATP in the presence of two divalent metal ions (Mn^{2+} or Mg^{2+}) (see Scheme 2). Subsequently the intermediate mevalonic acid 5-phosphate


C5 + C5
OPP
H
PPO
Geranyl - PP
Neryl PP
Monoterpenes
C10 + C5
Sesquiterpenes
(C15)
Farnesyl PP
2x
Squalene
C15 + C5
Diterpenes
(C20)
Geranyl geranyl PP
Sesterpenes
(C25)
C15 + C15
Triterpenes and Steroids
(C30)

Scheme 3

undergoes a second ATP phosphorylation to give mevalonic acid 5- pyrophosphate (**7**).[15] The latter compound is enzymatically transformed by ATP to IPP (**8**) with release of inorganic phosphate and carbon dioxide. Next the pyrophosphate (**8**) is isomerized to dimethylallyl pyrophosphate [DMAPP] (**9**) by an enzymatic process first elaborated by Lynen and co-workers.[20]



Scheme 2

Association of Five Carbon Units.

Most terpenoids are produced by aldol or Claisen condensations of IPP and DMAPP in a "head-to-tail" manner. The combination of these two molecules, with loss of a pyrophosphate ion from one of them and of a hydrogen ion from the other, leads to the formation of geranyl pyrophosphate (GPP). The initial product GPP is then transformed under various conditions to monoterpenes, sesquiterpenes, and the higher terpenes[4,7]. The formation of the acyclic terpenoids can be visualized as in Scheme 3. It is worth mentioning that most natural acyclic polyisoprenoids are "all-trans".[19]

Formation and Cyclization of Squalene.[17]

Squalene (**12**) the progenitor of triterpenoids and steroids, is derived from two farnesyl pyrophosphate (**10**) units joined in the unusual "head-to-head" fashion[16,17]. The stereochemistry of this process is known from tracer studies and is believed to proceed *via* the intermediate presqualene alcohol (**11**)[19]. A reasonable mechanism is shown in Scheme 4. The polycyclic structures formed from squalene can be rationalized in terms of the ways in which squalene or squalene oxide may be folded on the enzyme surface. For instance, the formation of euphol (**15**) (or tirucallol), the putative precursor of tetranortriterpenoids, involves cyclisation of squalene oxide in the chair-chair-chair-boat conformation (**13**) (see Scheme 5). The corresponding chair-boat-chair-boat folding of squalene oxide (**14**) leads to lanosterol (**16**), and hence the steroids. Cyclization is usually initiated by acid catalysed opening of squalene monoepoxide (**14**). Only the (3*S*)-enantiomer is used by a wide variety of biological systems[20].

MELIACEAE

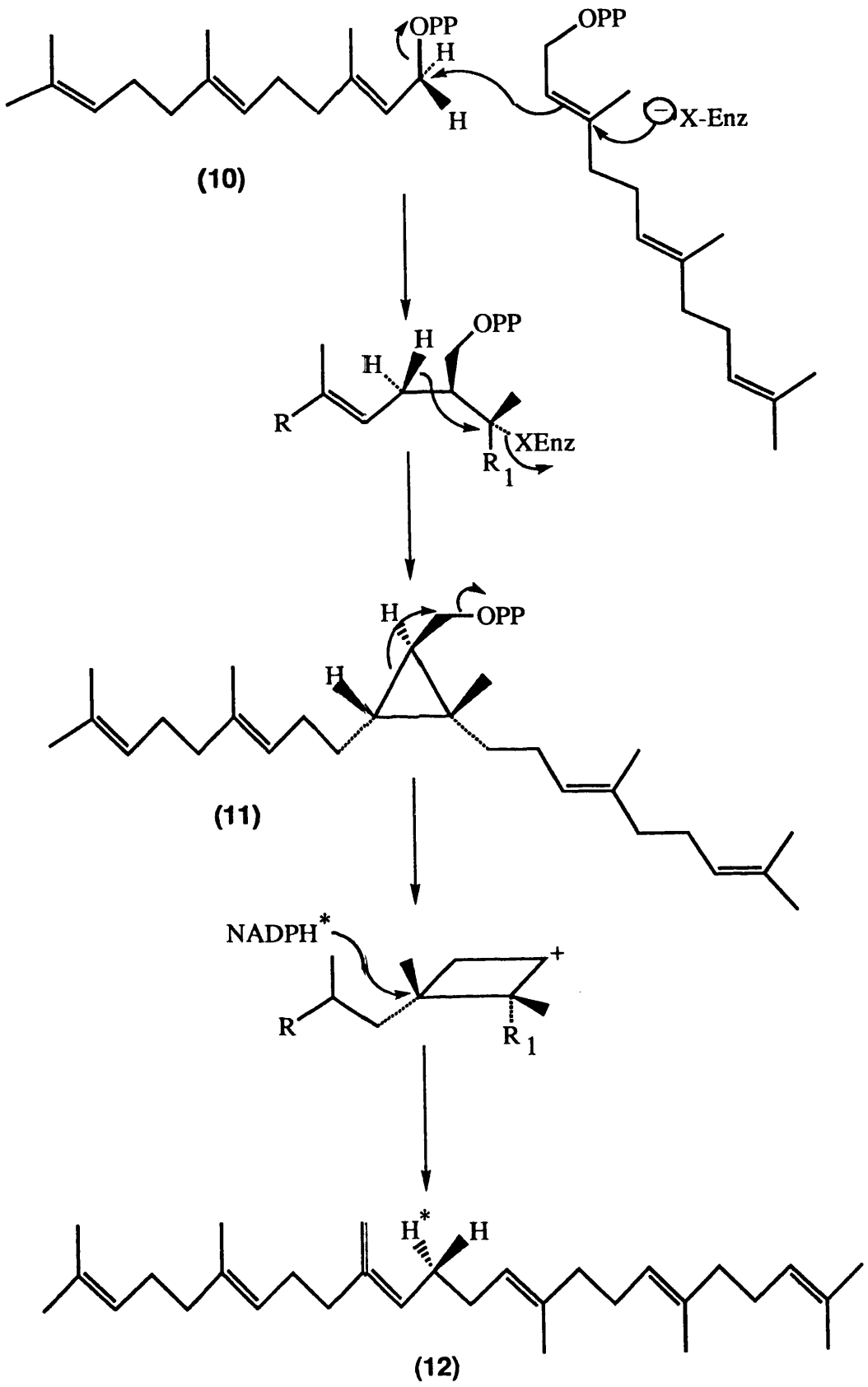
The Meliaceae family, from tropical and warm temperate regions, embraces many species and consists mostly of trees and shrubs. This family, which can be conveniently grouped into tribes, has commercial value and yields various woods such as Mahogany (*Swietenia*), African Mahogany (*Khaya*), Sapele Mahagony (*Entandrophragma*), and Spanish cedar (*Cedrela*). Sapele Mahagony is the most important commercial species and is used in the manufacture of plywood.[21] Some species have edible fruits and some contain useful oils. In India, the wood of *Cedrela toona* is largely used in making light furniture and musical instruments.[22]

Some plants of this family possess biologically active compounds which can be used for medicinal purposes. For instance, the bark of *Cedrela toona* is a powerful astringent and has been used for treating dysentery.[22,23] In Indonesia, *Toona surenii* is used as astringent and a tonic and is favoured for treating chronic diarrhea, dysentery, and other intestinal infections. Extracts of the *Toona surenii* leaves show antibiotic activity against *Staphylococcus.* The tincture of the bark of *Azadirachta indica*, well known in Indo-China, is useful for people with chronic malaria[23]. Jolad *et al* have reported, that some limonoids are active as anticancer agents.[24]

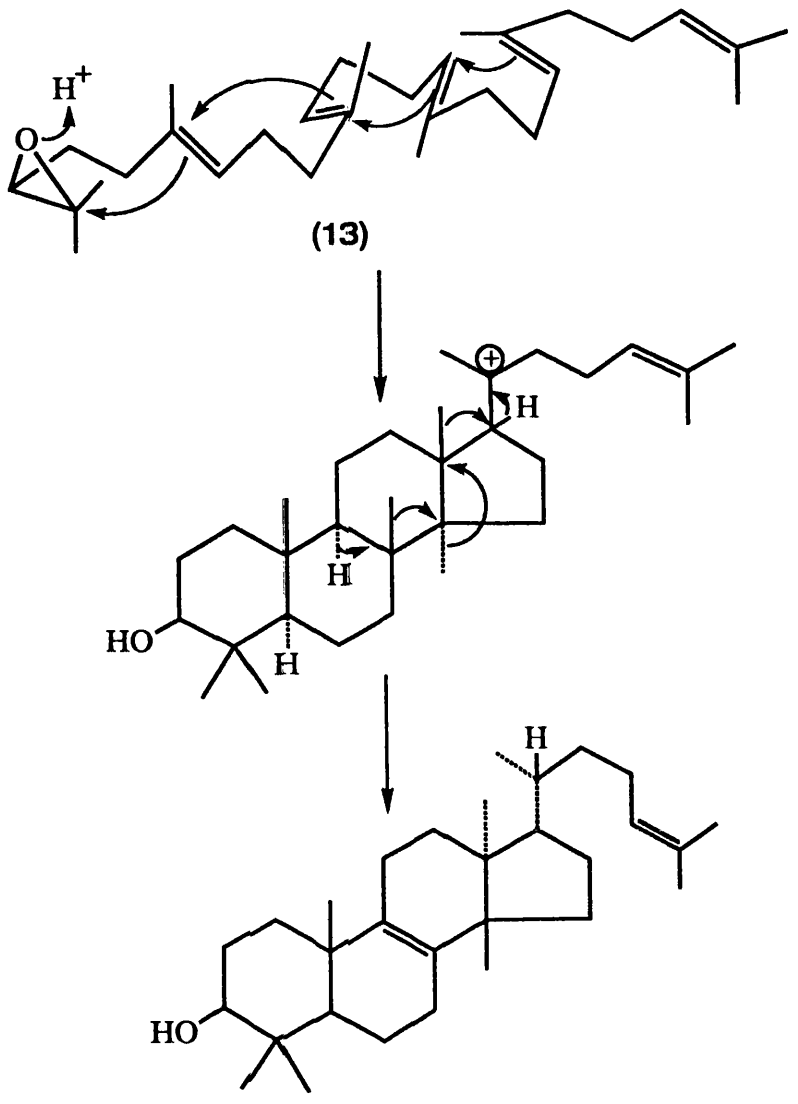
It is known that many species of Meliaceae contain limonoids e.g. azadirachtin which have been used for insect control in India[25]. Lavie *et al* in 1967 noted that limonoids of at least two species of the Meliaceae were active as insect antifeedants[26]. Limonoids are potentially available in very large quantities from the Meliaceae. Some species can produce 1% of an isolated crystalline limonoid. A single tree of *Entandrophragma angolense* may contain more than 100 kg of gedunin which is easily recoverable from timber mill off-cuts.

Most publications on the Meliaceae family concern the occurrence of limonoids. Taylor has noted that, in the Meliaceae, there is often appreciable variation between species and between populations and individuals in the amounts and types of limonoids that are produced. He recognized eleven classes of limonoids which are based on modification of Δ^7-triterpenes of the euphane or tirucallane type.[28]

Thus far, publications on the occurrence of sesquiterpenoids are limited. Nagasampagi *et al* in 1968 reported the isolation and partial synthesis of some sesquiterpenoids from the wood of *Cedrela toona* At least fourteen components including three new alcohols i.e, T-muurolol, epi-cubinol and cubenol were obtained.[29] The neem tree also contains diterpenoids.



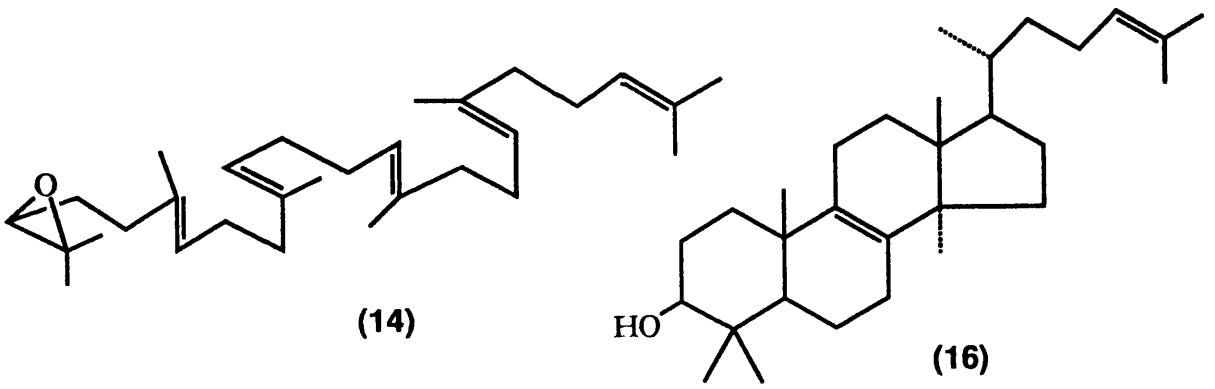
Scheme 4



(13)

(15)

Scheme 5



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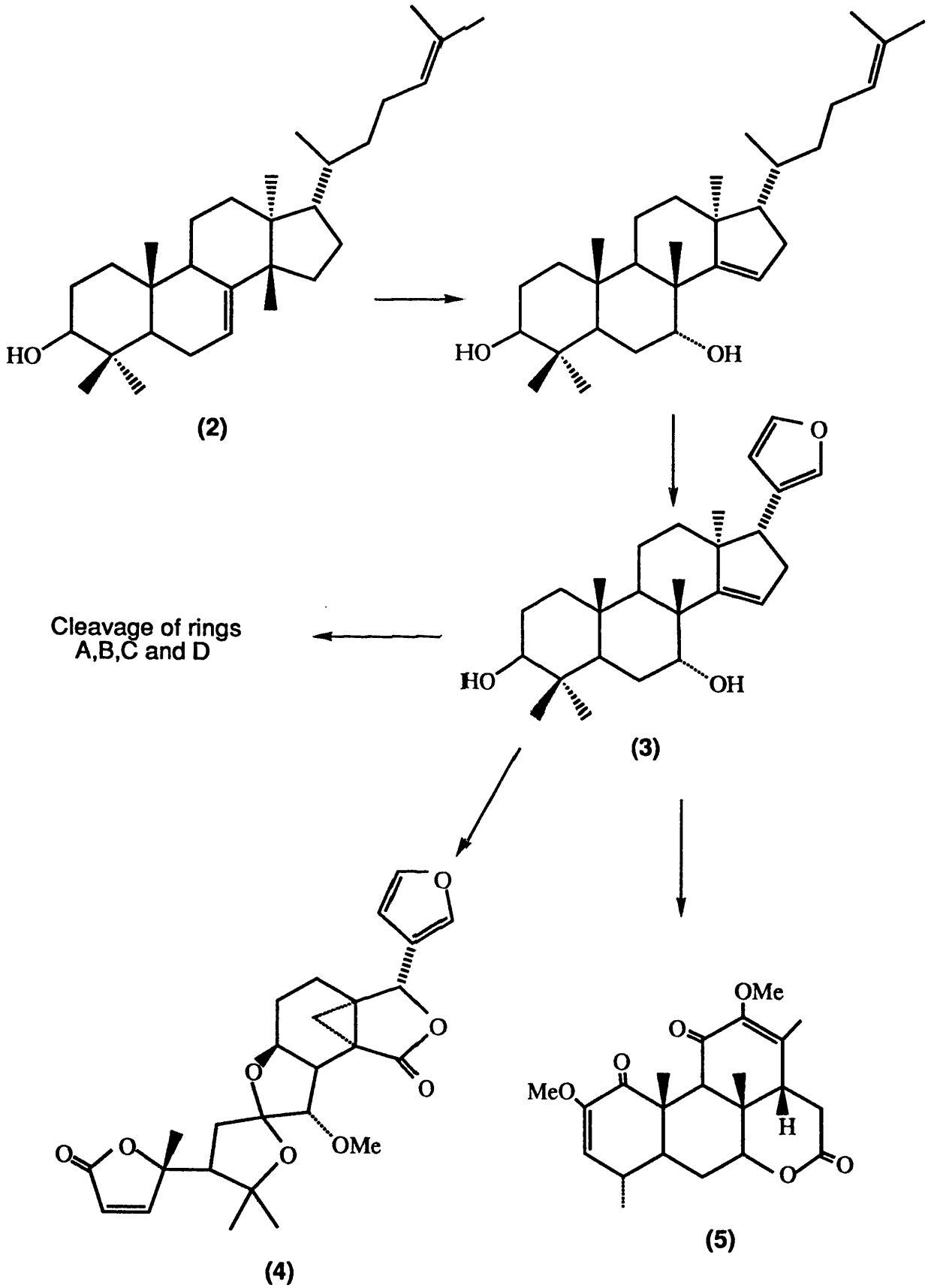
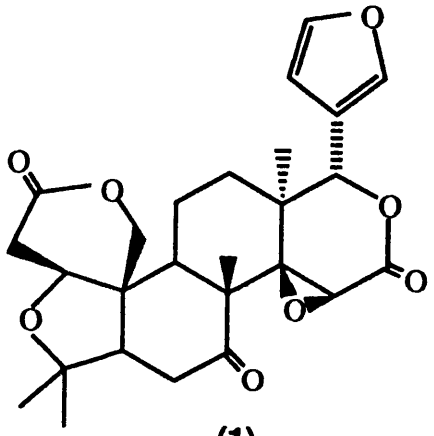
CHAPTER I

REVIEW OF TETRANORTRITERPENOIDS


HO
HO
OH
(2)
O
HO
OH
(3)
Cleavage of rings
A,B,C and D
O
O
O
O
O
O
OMe
O
(4)
OMe
O
O
MeO
H
O
O
(5)

Scheme 1

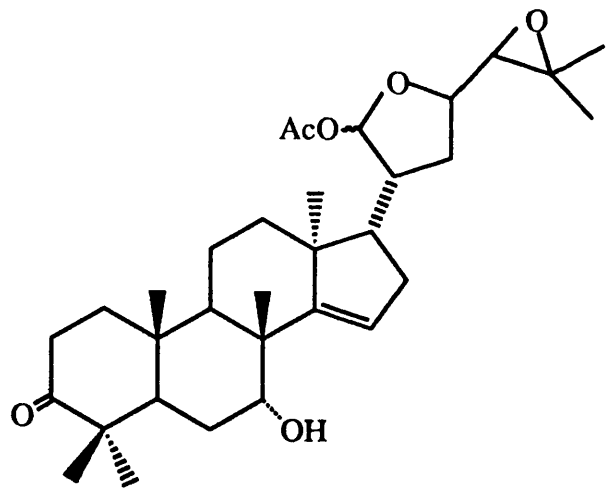
The tetranortriterpenoids form a large group of furanoid compounds found mainly in the Rutaceae, Cneoraceae, and Meliaceae.[1,2] The greatest structural diversity of tetranortriterpenoids occurs in the Meliaceae[2]. About 280 tetranortriterpenoids have been isolated from many different species of Meliaceae.[3] They are also called limonoids since their structures are related to limonin (**1**), which contains two lactone rings which can be opened reversibly, and a β - substituted furan ring.[4,6]



(1)

Biogenetically, tetranortriterpenoids may be derived from a euphol or tirucallol precursor (**2**) (Scheme 1), which first undergoes an *apo*-rearrangement with simultaneous introduction of oxygen at C-7 and then loss of the four terminal carbon atoms of the side chain with formation of the furan ring to give the simplest tetranortriterpenoid (**3**). Further oxidations, Baeyer-Villiger ring cleavages and rearrangements of (**3**), can lead to the wide variety of structural types which have been reported.[3,5]

It is interesting to note that (**3**) can suffer further degradation to give pentanortriterpenoids, e.g. (**4**) found in the Cneoraceae[7], and quassinoids, e.g (**5**) found in the Simaroubaceae.[1,8] While biosynthetic results for the proposed biogenesis of tetranortriterpenoids are limited, conclusive evidence has been obtained for the formation of quassinoids from mevalonate *via* a simple tirucallol precursor.[8]



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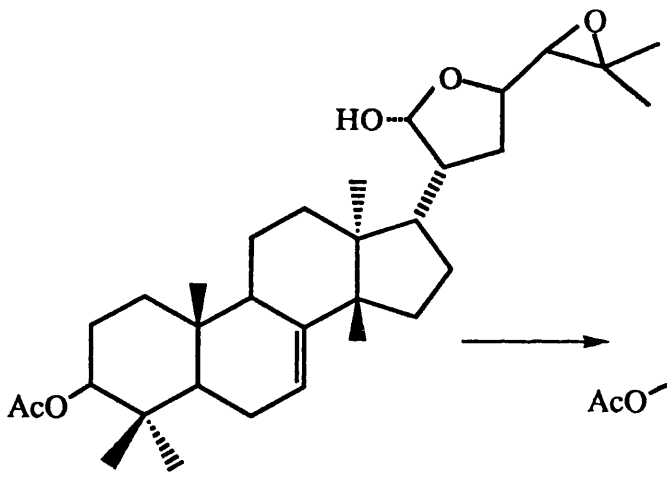

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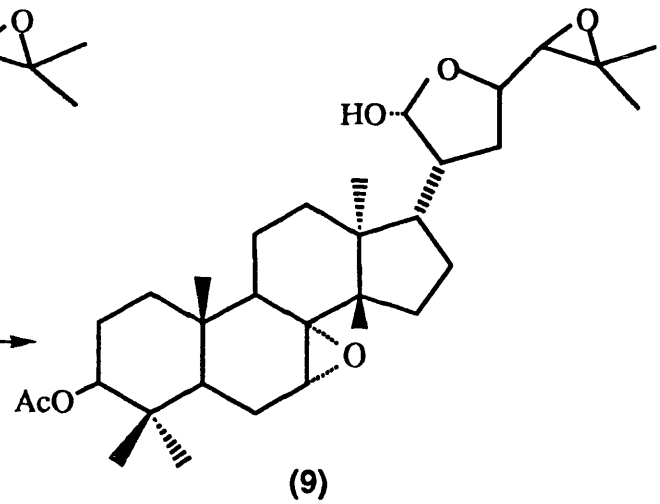

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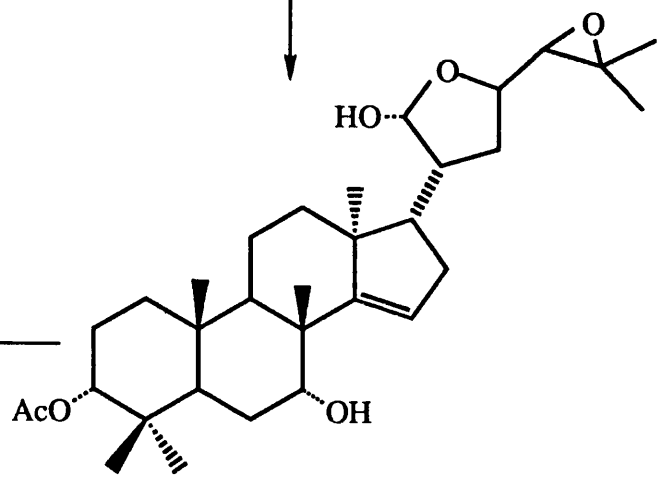

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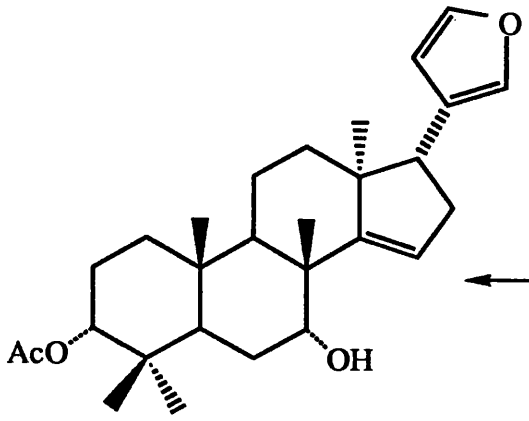
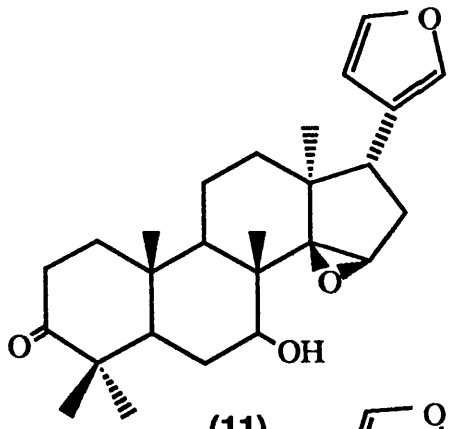


(8)

Scheme 2



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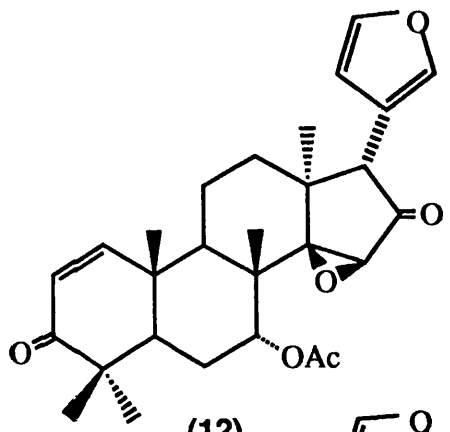

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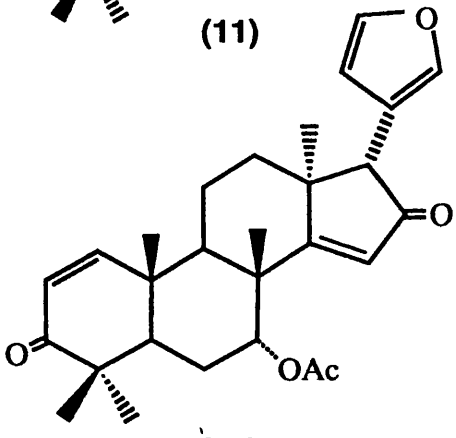

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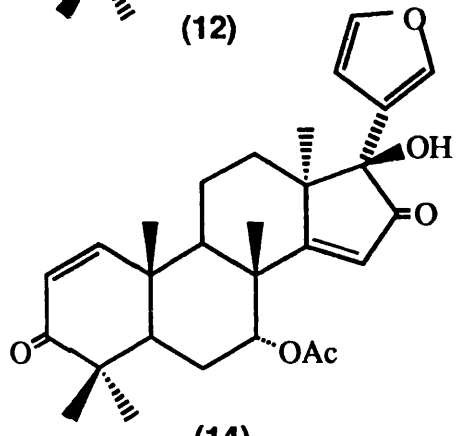

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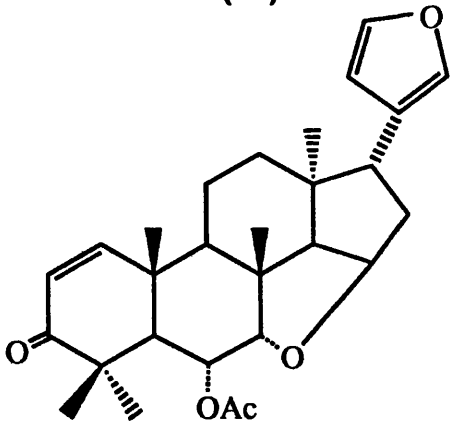

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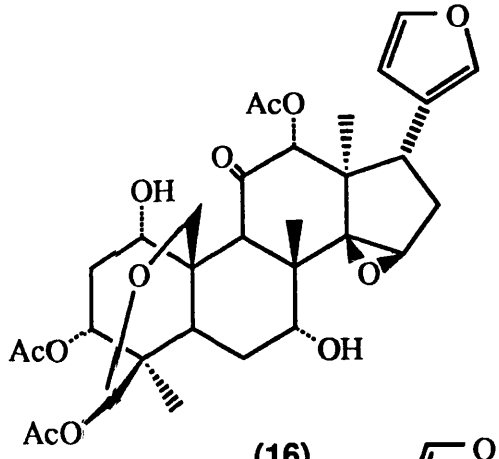

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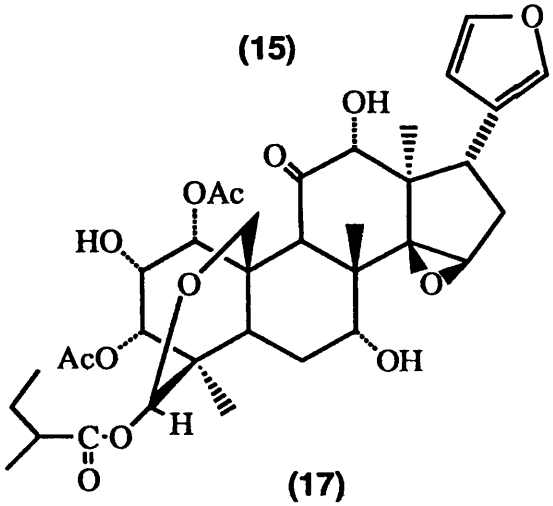

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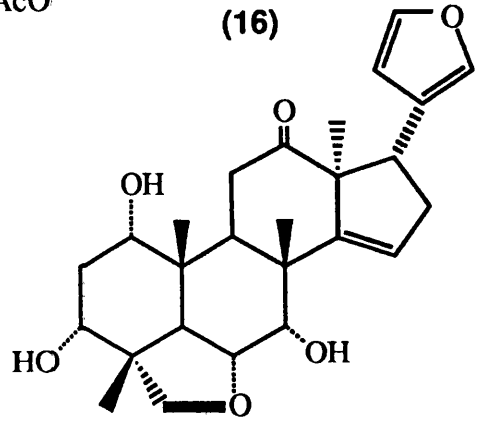
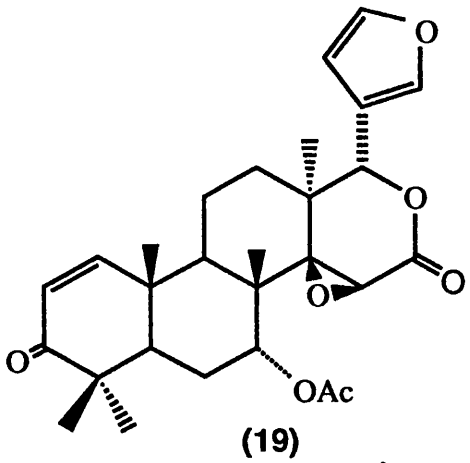


(18)

This brief review is intended to give a broad picture of the present state of knowledge in the tetranortriterpenoid series. It is convenient to consider these compounds in groups, according to the extent of ring cleavage. First, it is appropriate to discuss some of the C_{30} tirucallol and *apo*-tirucallol derivatives which often co-occur with the tetranortriterpenoids and which seem likely precursors.

(a) C_{30} Precursors.- Turreanthin (**6**)[9] is one example of the group of compounds with a pattern of side chain oxygenation which very probably represents an intermediate stage between tirucallol and the furan ring of tetranortriterpenoids. The isolation of *apo*-derivatives, including (**7**)[10], with an intact side chain suggests that skeletal rearrangement precedes furan formation. Halsall and his colleagues have demonstrated the possible intermediacy of these C_{30} compounds in the biogenesis of tetranortriterpenoids *in vitro* with a conversion of turreanthin into the simple limonoid (**8**) (see Scheme 2). Lewis acid treatment of the 7α, 8α -epoxide (**9**) smoothly converted it into the *apo*-derivative (**10**) with the desired oxygen substituent at C-7 and a 14, 15 - double bond.[11]

(b) Intact C_{26} - Skeleton.- At the stage of the simplest limonoid (**13**) further oxidations can occur in ring D giving rise to a variety of compounds with oxygen functions at carbons 14, 15, 16 and even 17. A common feature is epoxidation of the ring D double bond as in trichilenone (**11**)[12], sometimes accompanied by a ketonic carbonyl at C-16 as in nimbinin (**12**).[13] The isolation of azadiradione (**13**)[14] and other similar compounds [e.g. 17β - hydroxyazadiradione (**14**)[15]] suggests that functionalization of C-16 occurs prior to epoxidation of the double bond. Other oxidations can occur in rings A, B, and C at carbons 1, 2, 6, 11 and 12. Examples include vepinin (**15**)[16] with an ether between C-7 and C-15, and the highly oxygenated compounds sendanin (**16**)[17], with a hemiacetal between C-19 and C-29, and the related aphanastatin (**17**)[18], from *Aphanamixis grandifolia*, which has considerable antitumor activity. The presence of a hydroxyl group at C-6 may lead to the formation of an ether bridge with the 4α methyl group as in nimbidin (**18**).[19]



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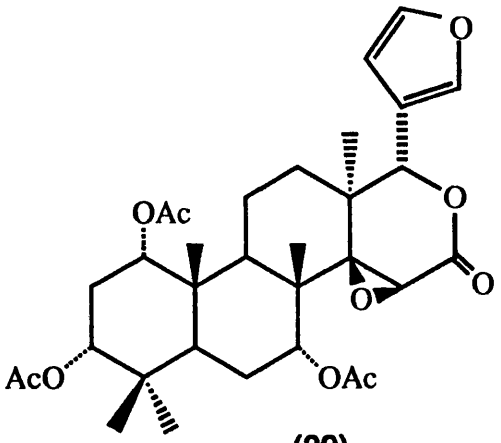

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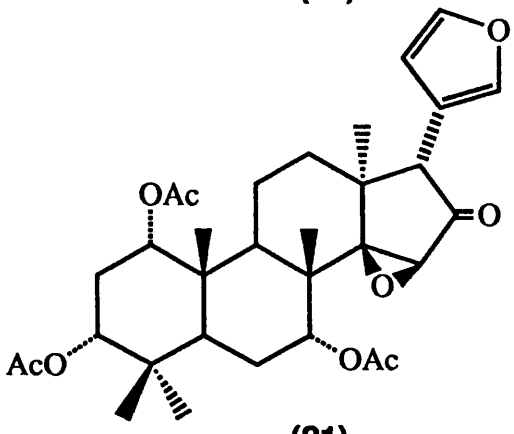

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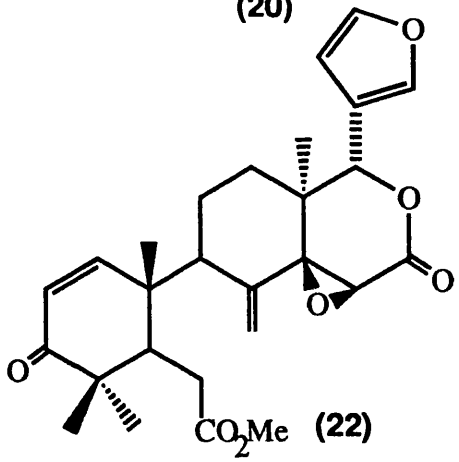

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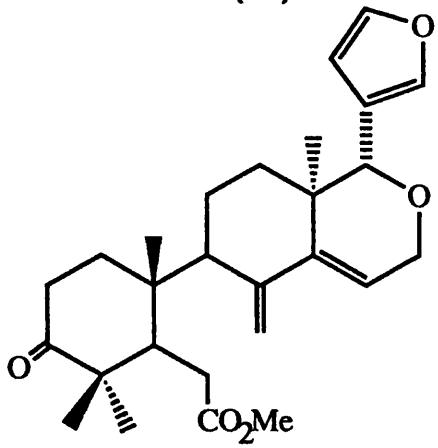

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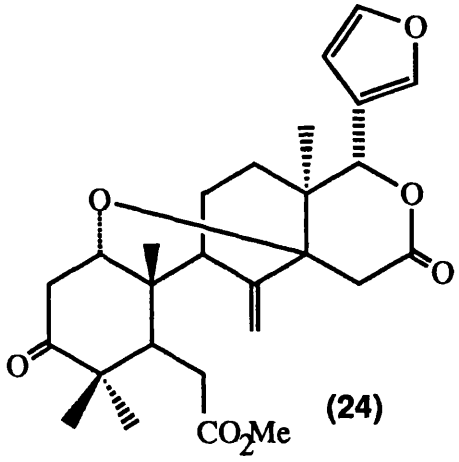

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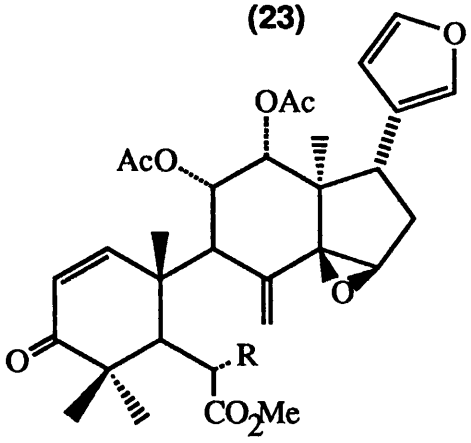
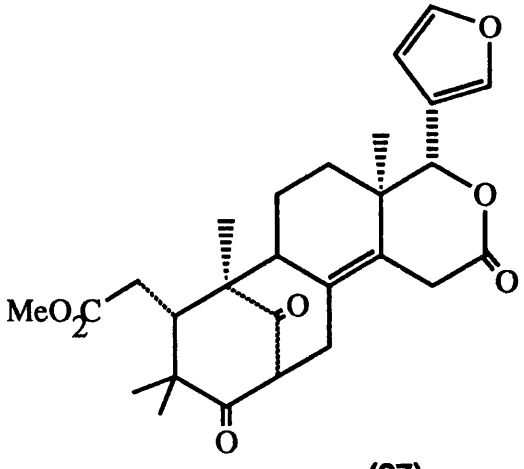


(25) R = H

(26) R = Ac



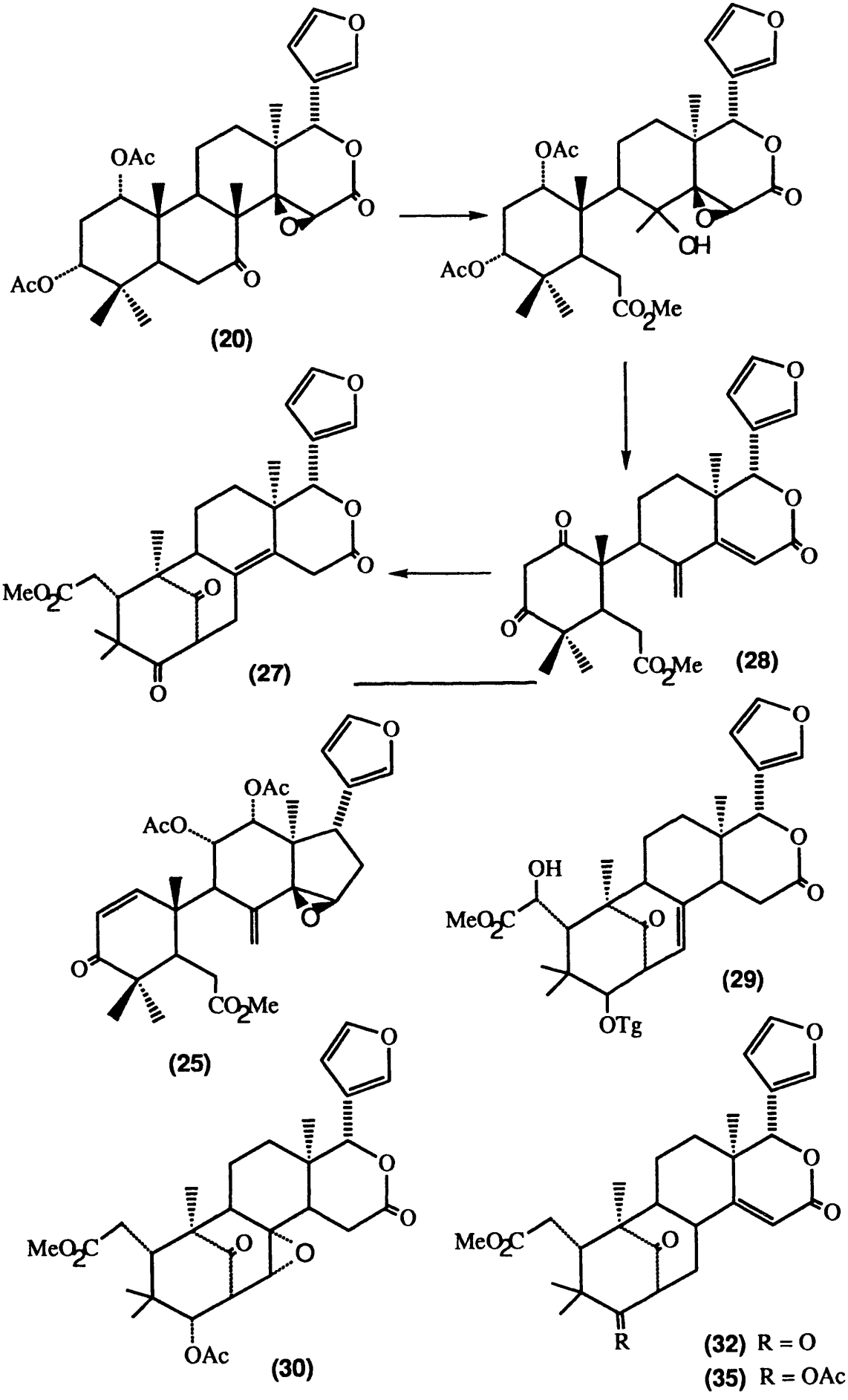
(27)

(c) Ring D Cleaved.- The next step in the elaboration of the tetranortriterpenoid skeleton leads to the formation of the characteristic ring D epoxylactone by biochemical Baeyer-Villiger oxidation of a 16 - oxo - precursor. Two of the most abundant tetranortriterpenoids gedunin (**19**)[20] and khivorin (**20**)[21], belong to this group. Both have been prepared *in vitro* by Baeyer-Villiger oxidation of the supposed precursors nimbinin (**12**) and khayanthone (**21**)[22,23] respectively. They often co-occur with complex ring B cleaved tetranortriterpenoids. It is therefore reasonable that compounds of this type represent an intermediate stage in their biosynthesis.

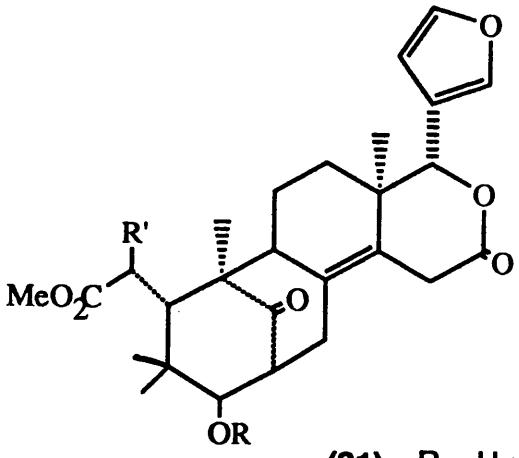
(d) Ring B Cleaved.- Many members of this group have also undergone cleavage of ring D. The typical ring B cleaved system, exemplified by andirobin (**22**)[24], can arise by formal Baeyer - Villiger oxidation of a 7-oxo compound followed by hydrolytic opening of the lactone and dehydration of the tertiary hydroxyl group to give the 8, 30 exomethylene group. The corresponding diene lactone, deoxyandirobin (**23**)[25] has also been isolated. Methyl angolensate (**24**)[26] has the interesting 1, 14-ether which presumably arises by addition of a 1α -hydroxyl group to the α, β - unsaturated ring D lactone. Both andirobin and methyl angolensate have been prepared *in vitro* by partial synthesis from khivorin (**20**).[27]

The first examples of simple ring B cleaved tetranortriterpenoids with an intact ring D are toonacilin (**25**) and its 6-acetoxy derivative (**26**) from *Toona ciliata*. They are of special interest because of their potent antifeedant activity against the Mexican bean beetle.[28]

This group of tetranortriterpenoids is unique in that the initial cleavage of ring B can be obscured by subsequent carbon-carbon bond formation between C-2 and C-30 to give the bicyclononanone ring system as in mexicanolide (**27**)[30]. An *in vitro* partial synthesis of mexicanolide from khivorin (**20**) has been achieved[27] (see Scheme 3). The diketone precursor (**28**) undergoes facile cyclisation in mild base to (**27**).

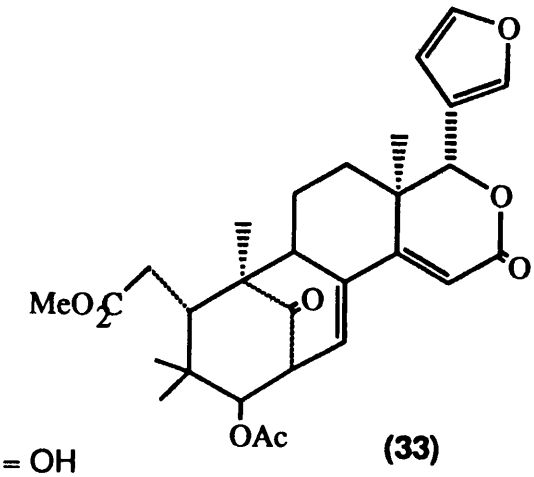

OAc
AcO
O
(20)
OAc
OH
AcO
CO2Me
MeO2C
(27)
(28)
AcO
OAc
CO2Me
(25)
OH
MeO2C
OTg
(29)
MeO2C
OAc
(30)
MeO2C
R
(32) R = O
(35) R = OAc

Scheme 3

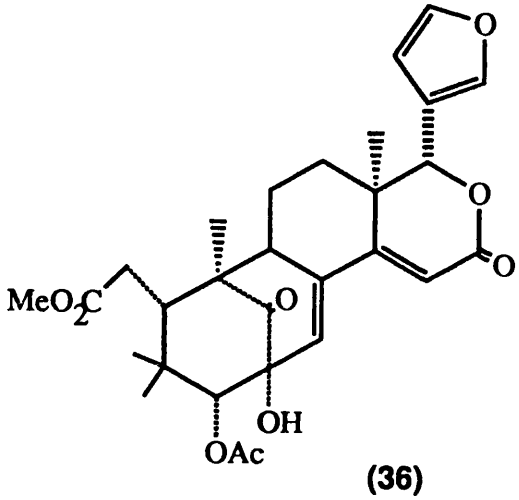


(31) R = H ; R' = OH

(34) R = Ac ; R' = H



(33)



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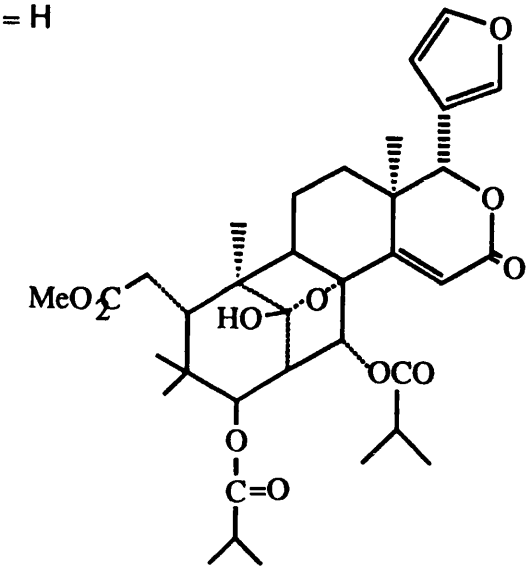

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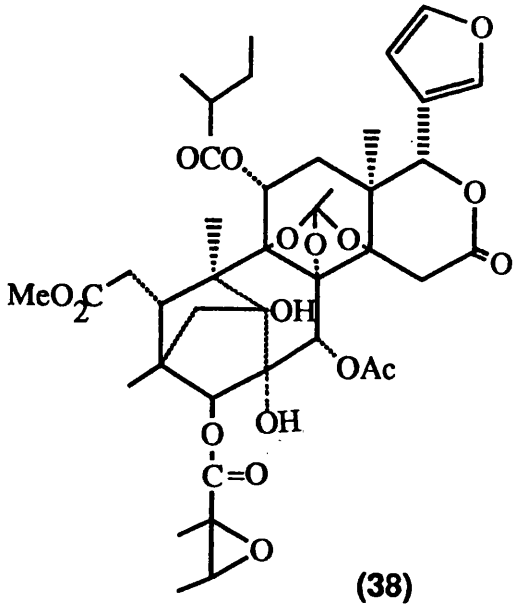

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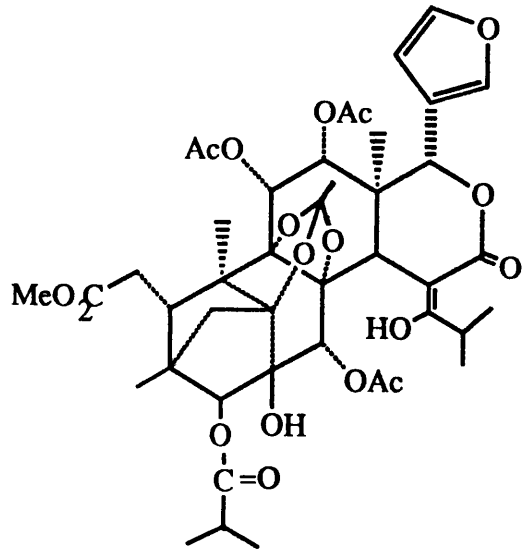


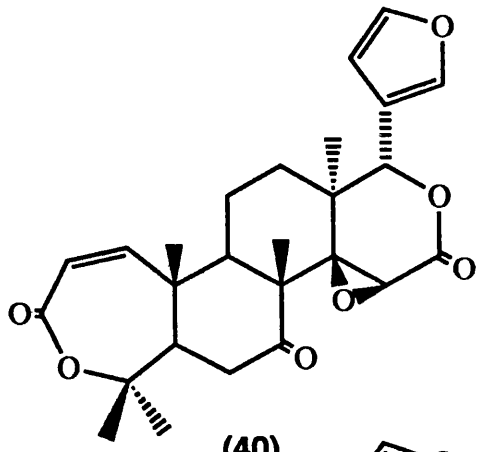
(39)

The first representative of this group is swietenine (**29**) from *Swietenia macrophylla*[31]. The residual 8, 30 double bond is very hindered and unreactive. Although the natural epoxide xylocarpin (**30**) has been isolated from *Xylocarpus granatum*[32], attempts to form the epoxide *in vitro* have been unsuccesful. The nuclear double bond is also found at 8,14 as in mexicanolide (**27**)[33] and swietenolide (**31**)[34] and at 14, 15 as in carapin (**32**)[15]. Angustadienolide (**33**) from *Cedrela angustifolia* is the corresponding diene. Hydrogenation afforded a mixture of fissinolide (**34**) and the carapin derivative (**35**).

Increasing oxidation level in this series is represented by 2α-hydroxyangustadienolide(**36**)[37] and xyloccensin A (**37**)[38] and leads to the highly complex compounds like utilin (**38**)[39] and bussein (**39**).[40] Two new features apparent in these structure are (a) the formation of a new carbocyclic ring between the 4α-methyl group and C-1; and (b) the introduction of the orthoacetate at 1, 8 and 9 or 8, 9 and 14. The reaction of an unactivated methyl group and a ketonic carbonyl is unusual and finds no analogy in photochemistry. The occurrence of compounds of this type is so far restricted to *Entandrophragma* and *Chukrasia* species - also in *Pseudocedrela*, *Capuronianthus*, *Xylocarpus* and *Soymida.*

(e) Ring A Cleaved.- Compounds in this group have the characteristic ring D epoxylactone system and many but not all come from *Citrus* species[14]. This group is of historic significance, since the development of the chemistry of the tetranortriterpenoids dates from the elucidation of the structure of limonin (**1**).[4] In limonin, the initial ring cleavage is obscured by subsequent reactions. The simple Baeyer-Villiger cleavage of ring A is more obvious in obacunone (**40**)[41] and nomilin (**41**)[42], two further compounds from *Citrus* species. Harrisonin (**42**)[43], from *Harrisonii abyssinica* has an interesting hemiacetal function at C-7.

Nomilinic acid (**43**)[44] represents the opened form of the ring A ε-lactone and may be regarded as a precursor of the C-19 oxidised derivatives inchangin (**44**) and limonin (**1**). Veprisone(**45**)[46] is a simpler example of the C-1, C-4 ether which is probably formed by addition of the



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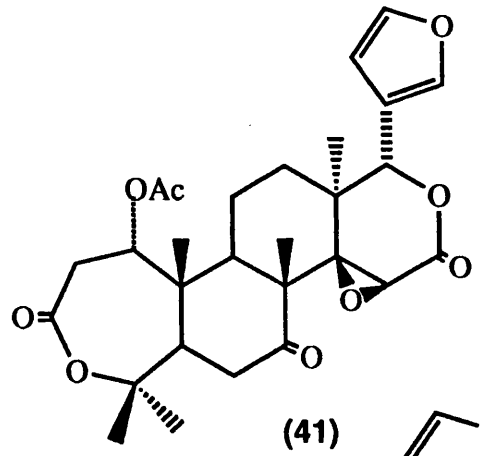

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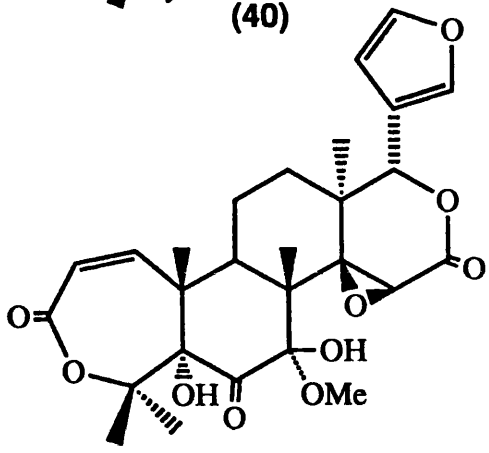

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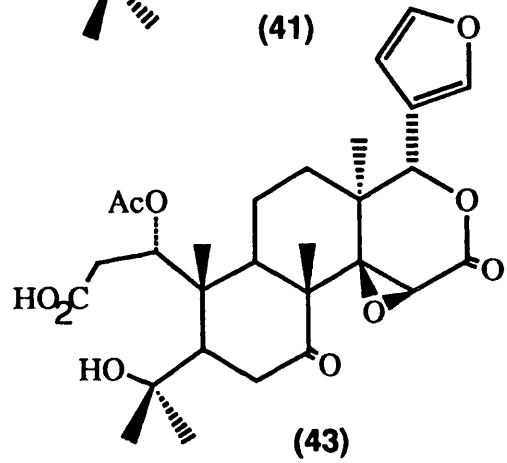

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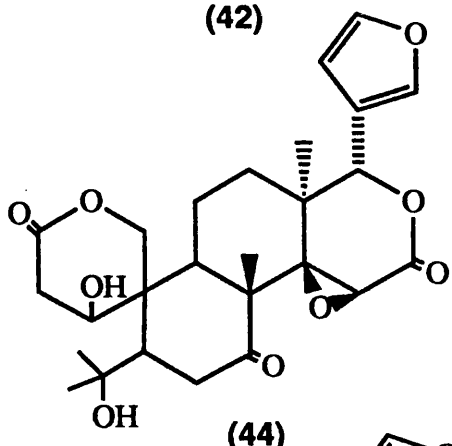

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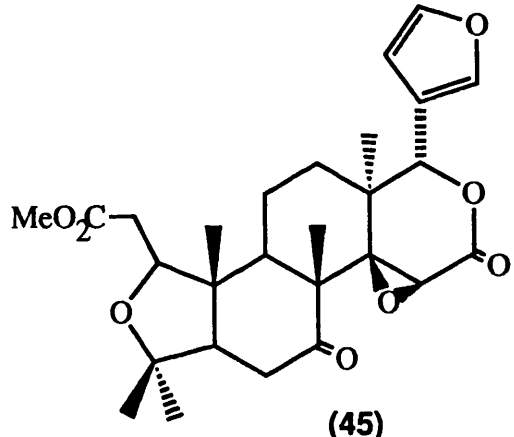

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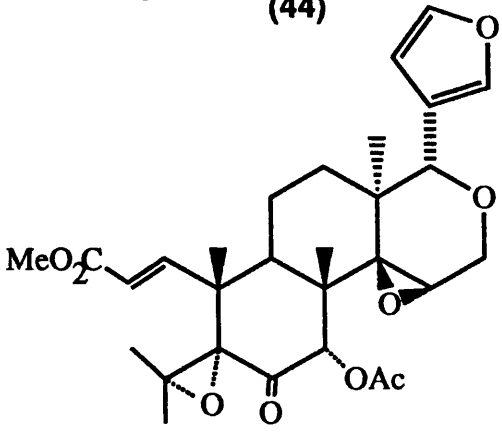

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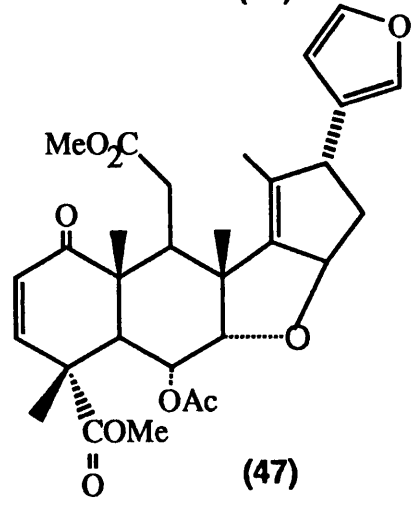

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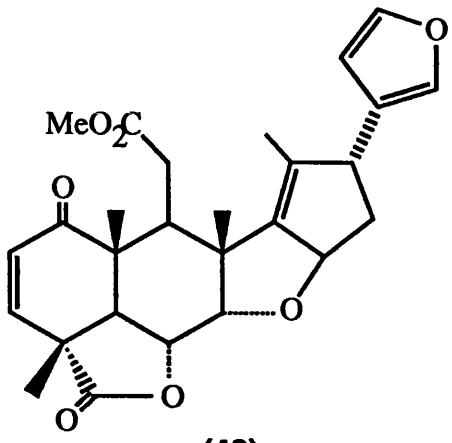

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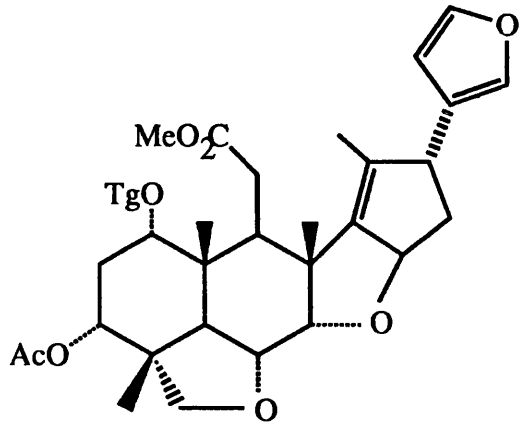

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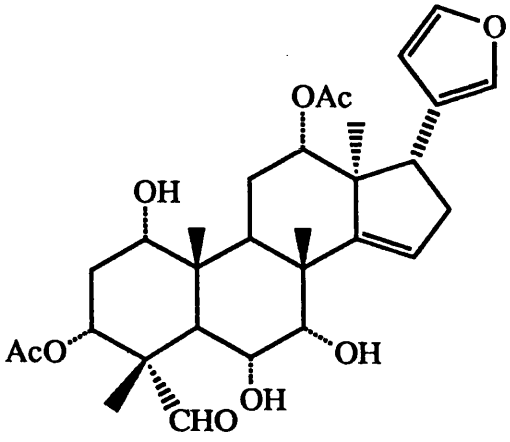

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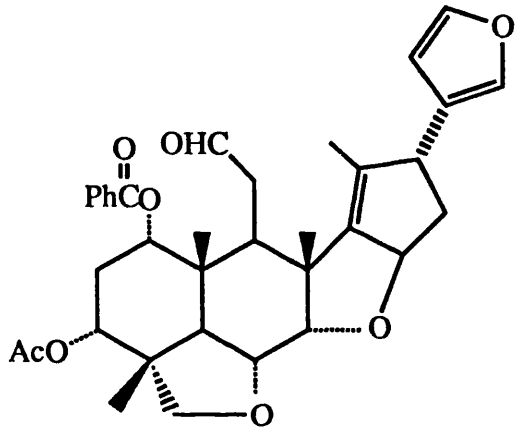

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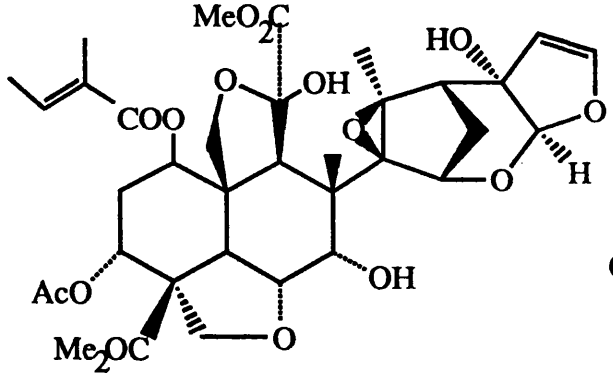

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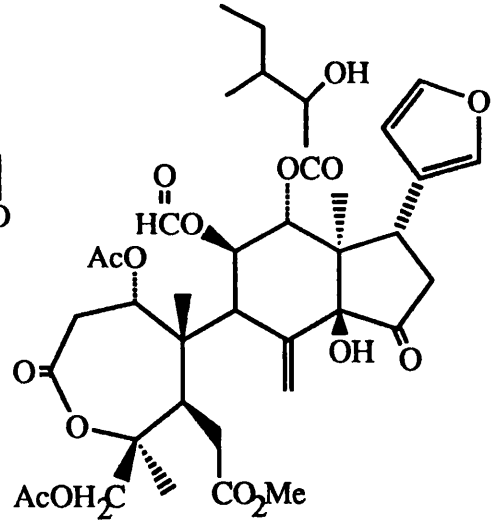

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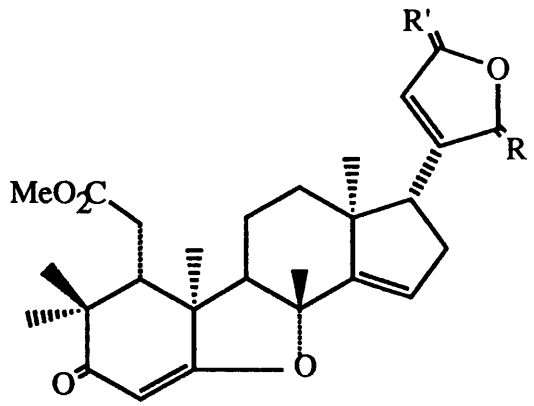
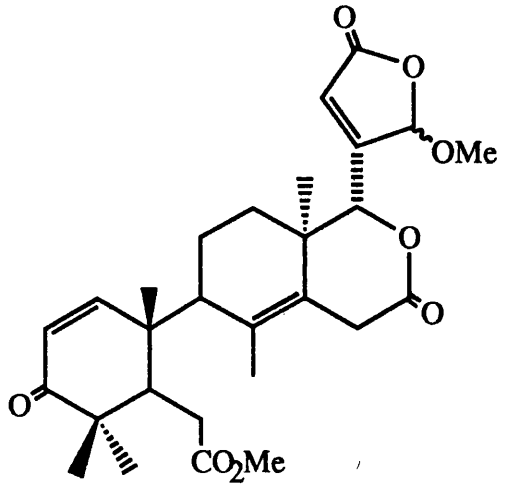


(54) R = O ; R' = OH

(55) R = OH ; R' = O



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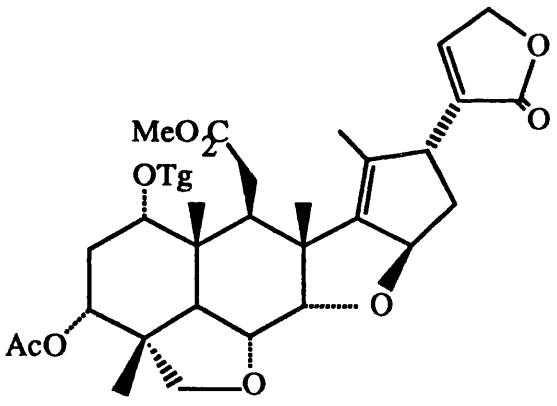

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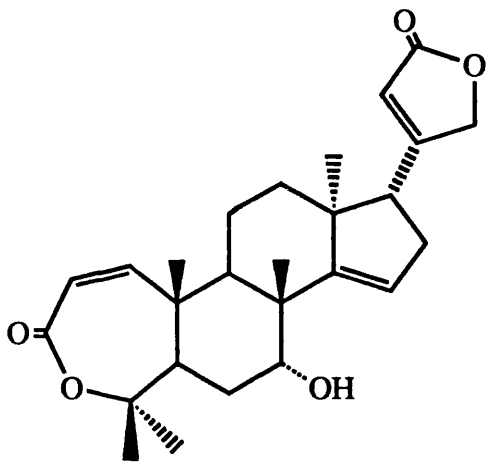

(58)

C-4 tertiary hydroxyl group to the unsaturated ester [or lactone as in limonin (**1**)]. Alternatively, dehydration and epoxidation leads to spathelin (**46**).[47]

(f) Ring C Cleaved.- This group of compounds is restricted to *Melia azedarach* and *Azadirachta indica*[1]. Nimbin (**47**)[48], nimbolide (**48**)[49] and salannin (**49**)[50] illustrate the common features. Sendanal (**50**)[51], isolated from *M. azedarach*, has the appropriate functionality for transformation into the above compounds. Ohchinol (**51**)[52] is biogenetically interesting since it represents simple ring C cleavage of a 12-hydroxy precursor [e.g. sendanal (**50**)]. The most interesting and most complex member of this group is azadirachtin (**52**)[53], a powerful locust antifeedant.

(g) Rings A and B Cleaved.- The first member of this interesting group is prieurianin (**53**) from *Trichilia prieuriana*. Other members of this group, isolated from *Trichilia* and *Guarea* species have also been reported[54].

(h) Modified Side Chains.- A growing number of tetranortriterpenoids with the modification of the usual furan ring have appeared recently. These include the isomeric γ-hydroxybutenolides (**54**) and (**55**)[55], the methoxybutenolide (**56**)[56], the butenolide (**57**)[57] and the γ-lactone (**58**)[58]. It is uncertain whether all these compounds are genuine natural products or artefacts formed by the action of light and oxygen on the furan ring. Photooxidation of several tetranortriterpenoids has been shown to give the corresponding γ-hydroxy butenolides.[59]

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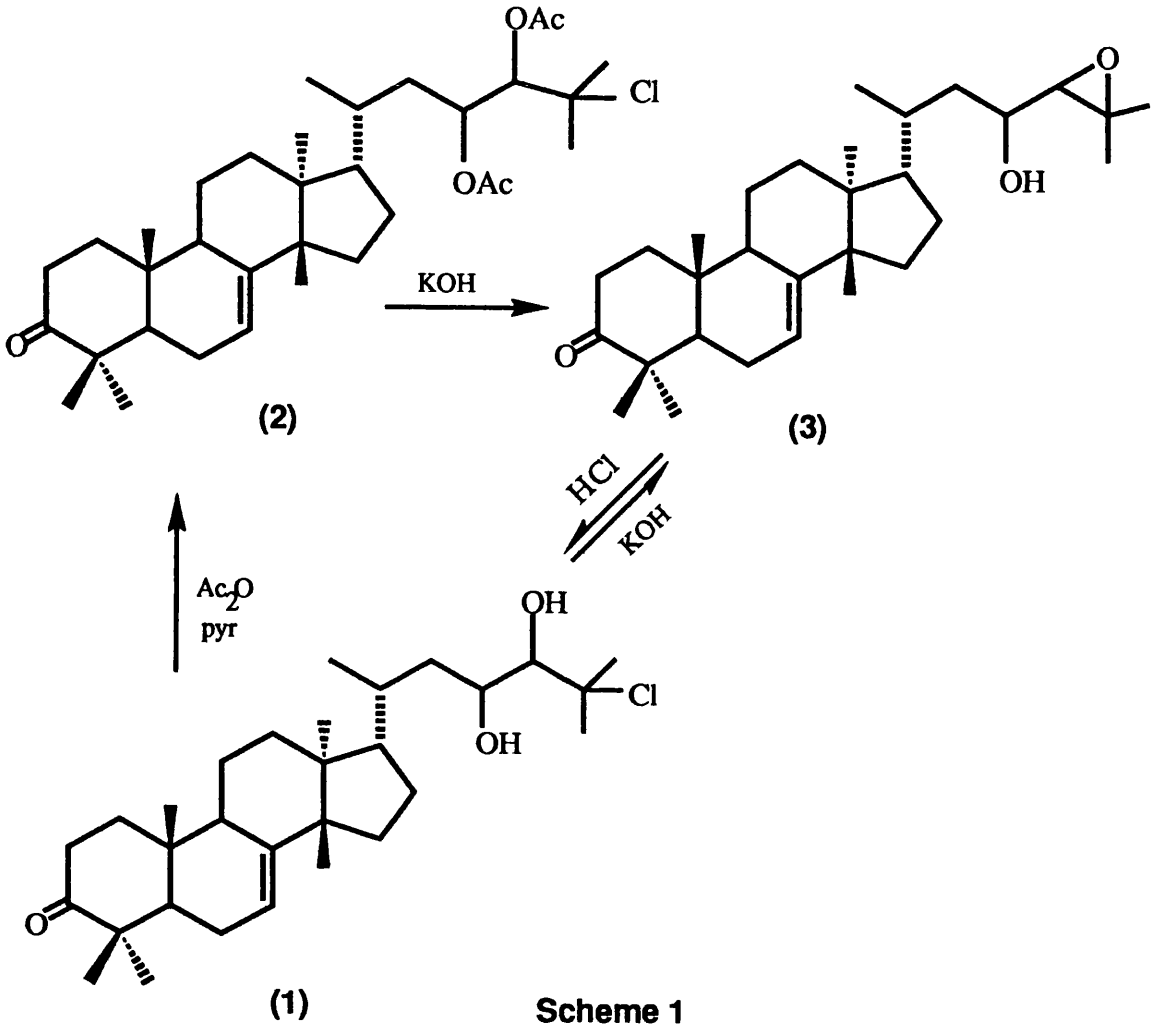
CHAPTER II

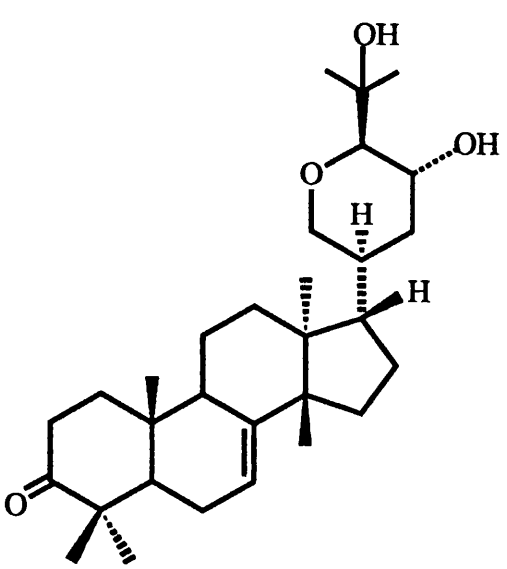
VAVAEA AMICORUM

INTRODUCTION

This chapter is concerned with the isolation of triterpenoids from the stem bark of *Vavaea amicorum* (Meliaceae) collected in Australia. *Vavaea* is the only genus found in the Vavaeeae tribe, subfamily of Melioideae.[1] Morphologically, *Vavaea* is rather isolated within the Melioideae, and is best placed between the tribe Turraeeae and Trichilieae, but is more closely related to the latter. The genus *Vavaea* ranges from Sumatra to Fiji and Tonga[2-4]. So far the chemical constituents of *Vavaea* have not been investigated. No economic use of this tree or shrub has been reported.



Scheme 1



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DISCUSSION

Chromatography of the ethyl acetate extract of the stem bark of *Vavaea amicorum* over silica gel, followed by preparative TLC of combined fractions, afforded β-sitosterol and the tirucallane derivatives bourjotinolone A (**4**) and the epoxide (**3**) related to bourjotinolone C (**1**) [Identical with niloticin p.30]. No limonoids were detected.

The epoxide (**3**), $C_{30}H_{48}O_3$, was obtained as a gum. Its ^1H NMR spectrum (Fig.1) showed peaks for one secondary and seven tertiary methyl groups [δ_H 0.93 (d, J 5.8 Hz), 0.79, 0.98, 1.00, 1.02, 1.09, 1.30, 1.31] a vinyl proton [δ_H 5.28 (dt, J 2.8, 3.2 Hz] and a secondary carbinol [δ_H 3.55 (dt, J 5.2, 8.3 Hz)] coupled to an trisubstituted epoxide proton [δ_H 2.63 (d, J 8.3 Hz)]. The presence of two deshielded methyls (δ_H 1.30, 1.31) indicated that the epoxide was situated at the end of a typical tetracyclic triterpenoid side-chain . The ^{13}C NMR spectrum of the epoxide (**3**) is presented in Fig. 2. and the data are listed in Table 1 . The resonance at δ_C 216.9 revealed an isolated ketone presumably situated at C-3 in addition to the trisubstituted double bond, secondary carbinol and trisubstituted epoxide. The above functionalities are readily assembled to give the structure (**3**) for the epoxide. This compound has not been isolated previously from nature but has been synthesised from bourjotinolone C (**1**)[see Scheme 1].[5]

Preparative TLC of later fractions afforded two bands, one of which was still clearly a mixture of tetracyclic triterpenoids. Further efforts at separation of this mixture failed to yield any pure compounds. The other band gave a pure compound $C_{30}H_{48}O_4$ as a gum. It had signals in its ^1H NMR spectrum (see Fig. 3) for seven tertiary methyl groups [δ_H 0.75, 0.97, 1.01 (2), 1.08, 1.24 and 1.28] two of which are associated with a carbon bearing oxygen, a vinyl proton [δ_H 5.30 (m)] and four other protons attached to carbon bearing oxygen [δ_H 3.38, 3.92 and 4.10]. The ^{13}C NMR spectrum (see Fig. 4 and Table 1) suggested the prsence of an isolated ketonic carbonyl, a

trisubstituted double bond and four carbons bearing oxygen, two secondary, one primary and one tertiary. These data fit well for structure (**4**) which is bourjotinolone A. Comparison with published data confirmed its identity.[6,7]

It is of taxonomic interest that *Vavaea amicorum* should produce tirucallane derivatives, the precursors of tetranortriterpenoids. No limonoids were detected in this investigation of the bark. It would obviously be of interest to examine the seed, leaves and wood.

GENERAL EXPERIMENTAL

All solvents used in the extractions and chromatographic separations were distilled. Petroleum ether refers to the fraction of b.p. 40°- 60°C.

Melting points were taken on a Kofler hot stage equipped with microscope and are uncorrected. IR spectra were recorded (in CCl_4) on a Perkin-Elmer model 727 B spectrometer. ^1H NMR measurements were made in $CDCl_3$ solution, on 90 M Hz Perkin Elmer and Bruker WP 200 SY instruments with TMS as an internal standard at δ 0 or $CHCl_3$ at δ 7.25 and all signals are reported as δ values. ^{13}C NMR spectra were obtained at 50 M Hz with $CDCl_3$ as an internal standard at δ 77.0. The chemical shifts are reported in ppm downfield from TMS. All 2D spectra have been recorded on a Bruker AC 300 instrument. The HMBC and HMQC data were sets of 256 FIDs of 2 K points. Direct and long-range coupling constants have been choosen equal to 135 Hz and 7 Hz respectively. Mass spectra were determined on A.E.I.-G.E.C MS-12 mass spectrometer, high resolution spectra being obtained on an A.E.I.- MS-902S instrument. Mass spectra were also obtained with a Hewlet-Packard 5880-A (FID detector) combined with a Varian 1400 chromatograph coupled to a Varian MAT CH-7A spectrometer, using column of CP sil 5 CB (25 m x 0.32 mm x 0.12 μm).

Flash column chromatography was conducted on Merck silica gel 60 GF_{254} (70-230 mesh). Preparative TLC utilized silica gel 60 GF_{254} coated to 1 mm thickness and viewed under UV light (254 or 366 nm). TLC of compounds was performed on Merck silica gel GF_{254} and the spots were visualized under UV light or by spraying with 25% sulfuric acid followed by heating (110°C, 5 min).

EXPERIMENTAL

The dried ground stem bark (370 g) was extracted first with ethyl acetate and then with methanol. Evaporation of the ethyl acetate extract *in vacuo*, yielded an oily dark green residue (9.04 g). The dried extract was redissolved in small volume of ether and chromatographed on a dry column of silica gel GF_{254} (100 g). Initial elution was with petroleum ether followed by increasing proportions of ethyl acetate in petroleum ether and then with increasing proportions of methanol in ethyl acetate. A total of 58 fractions(50 ml each) were collected.

The fractions were chromatographed on analytical silica gel GF_{254} TLC plates for evaluation and grouping. Combined fractions 26, 27 and 28 (M7) were subjected to preparative TLC using petroleum ether-ethyl acetate (70-30) as solvent gave 9 bands. Band 5 (M7B5) afforded a gum (31 mg) whose spectroscopic properties (Table 1 and Figure 1 and 2) indicated epoxide (**3**).

Preparative chromatography of combined fractions 29 and 30 (M8) on silica gel using petroleum ether-ethyl acetate (70-30) followed by petroleum ether-ethyl acetate (80-20) as solvents gave 7 bands. NMR analysis of band 6 (M8B6), a gum, revealed that it was bourjotinolone A (**4**). Its spectroscopic properties are shown in Table 1 and Figure 3 and 4.

The only other resonable band 5 (M8B5) proved to be an inseparable mixture of compounds similar to bourjotinolone. The difficulty of the separation arises if the mixture is in equilibrium.[8] The limited amount of material prevented any further work.

Table 1. 13C NMR chemical shifts of compound (**3**) and (**4**) and its reference[6] in $CDCl_3$.

Carbon	δ_C of (**3**), mult	δ_C of (**4**) mult.	
	Experimental	Experimental	Reference
1	38.5 t	38.5 t	38.5 t
2	34.9 t	34.9 t	34.9 t
3	216.9 s	216.9 s	217.1 s
4	47.8 s	47.8 s	47.9 s
5	52.3 d	52.3 d	52.4 d
6	24.3 t	24.3 t	24.3 t
7	117.9 d	118.0 d	118.1 d
8	145.7 s	145.6 s	145.7 s
9	48.4 d	48.4 d	48.4 d
10	35.0 s	35.0 s	34.9 s
11	18.2 t	18.1 t	18.2 t
12	33.5 t	32.9 t	32.9 t
13	43.5 s	43.3 s	43.2 s
14	51.2 s	51.3 s	51.2 s
15	34.0 t	33.9 t	33.9 t
16	28.7 t	27.4 t	27.4 t
17	53.2 d	44.7 d	44.7 d
18	12.7 q	12.8 q	12.8 q
19	21.5 q	21.6 q	21.6 q
20	33.5 d	37.4 d	37.5 d
21	19.8 q	70.0 t	70.1 t
22	40.6 t	36.3 t	36.4 t
23	68.5 d	64.6 d	64.6 d
24	69.2 d	86.4 d	86.4 d
25	60.3 s	74.2 s	74.1 s
26	19.8 q	23.9 q	23.9 q
27	24.8 q	28.5 q	28.4 q
28	27.3 q	27.4 q	27.4 q
29	24.5 q	24.5 q	24.5 q
30	21.7 q	22.3 q	22.3 q

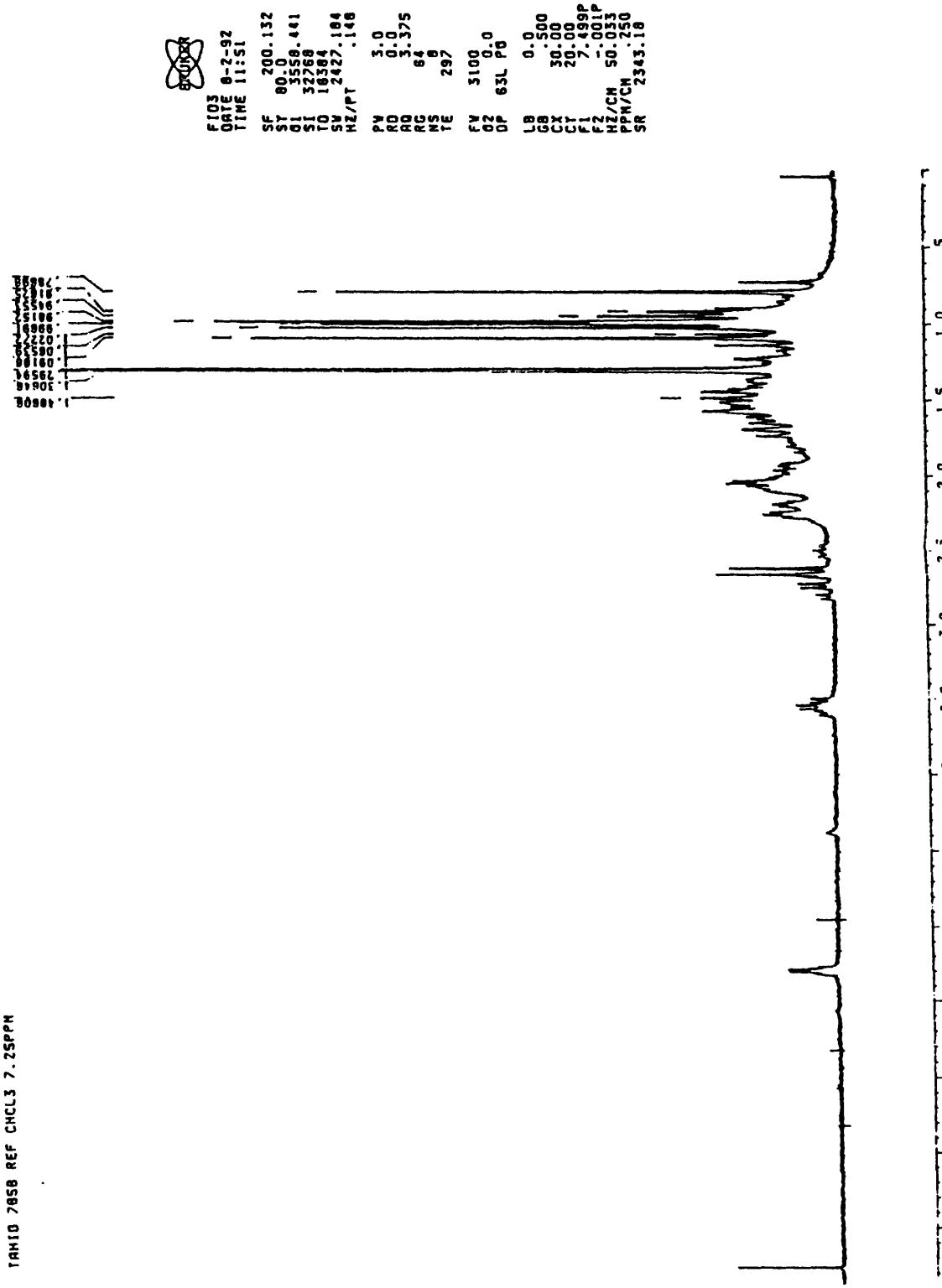

Figure 1. ^{1}H NMR spectrum of compound (3) in $CDCl_3$.

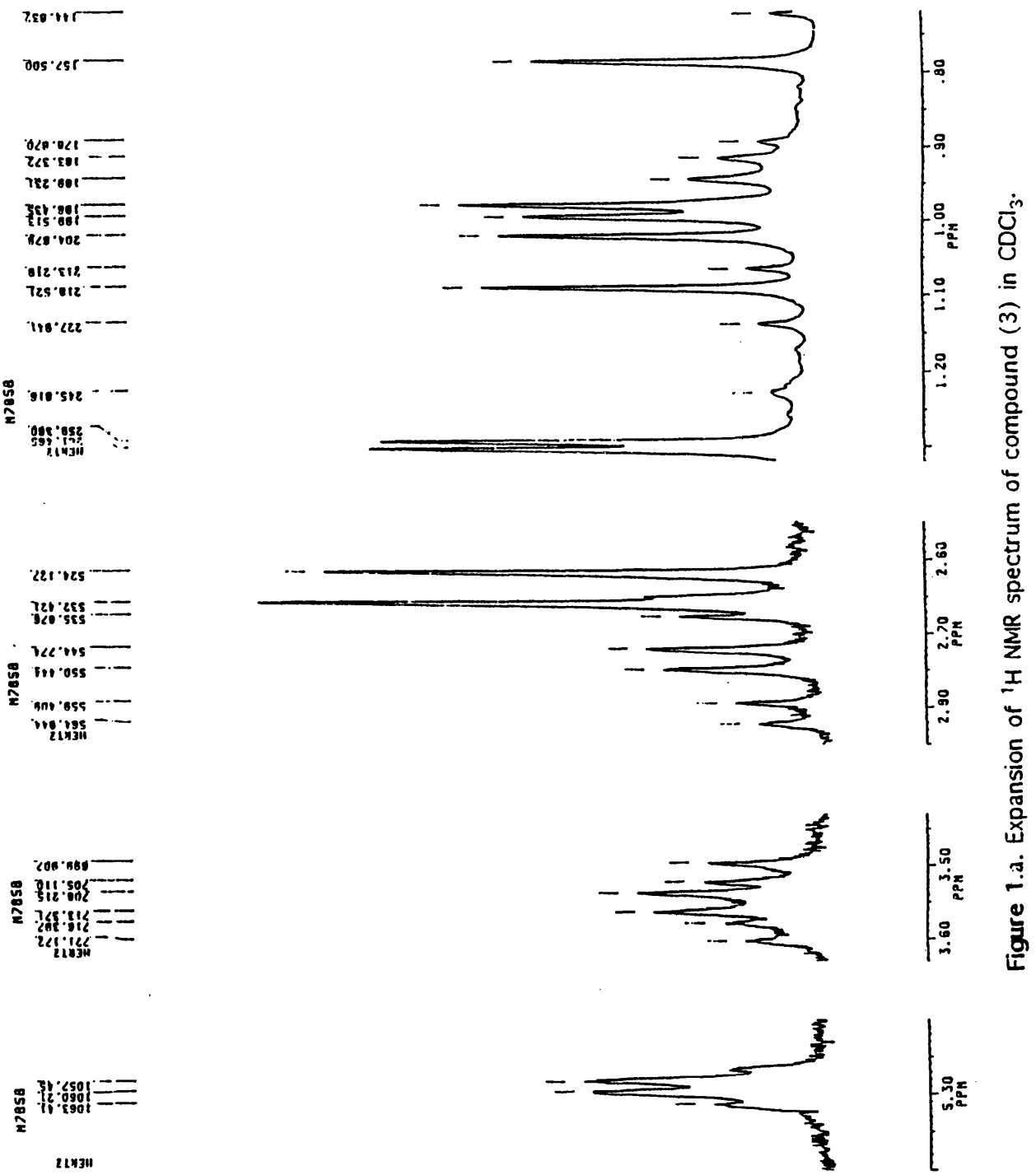

Figure 1.a. Expansion of ^1H NMR spectrum of compound (3) in $CDCl_3$.

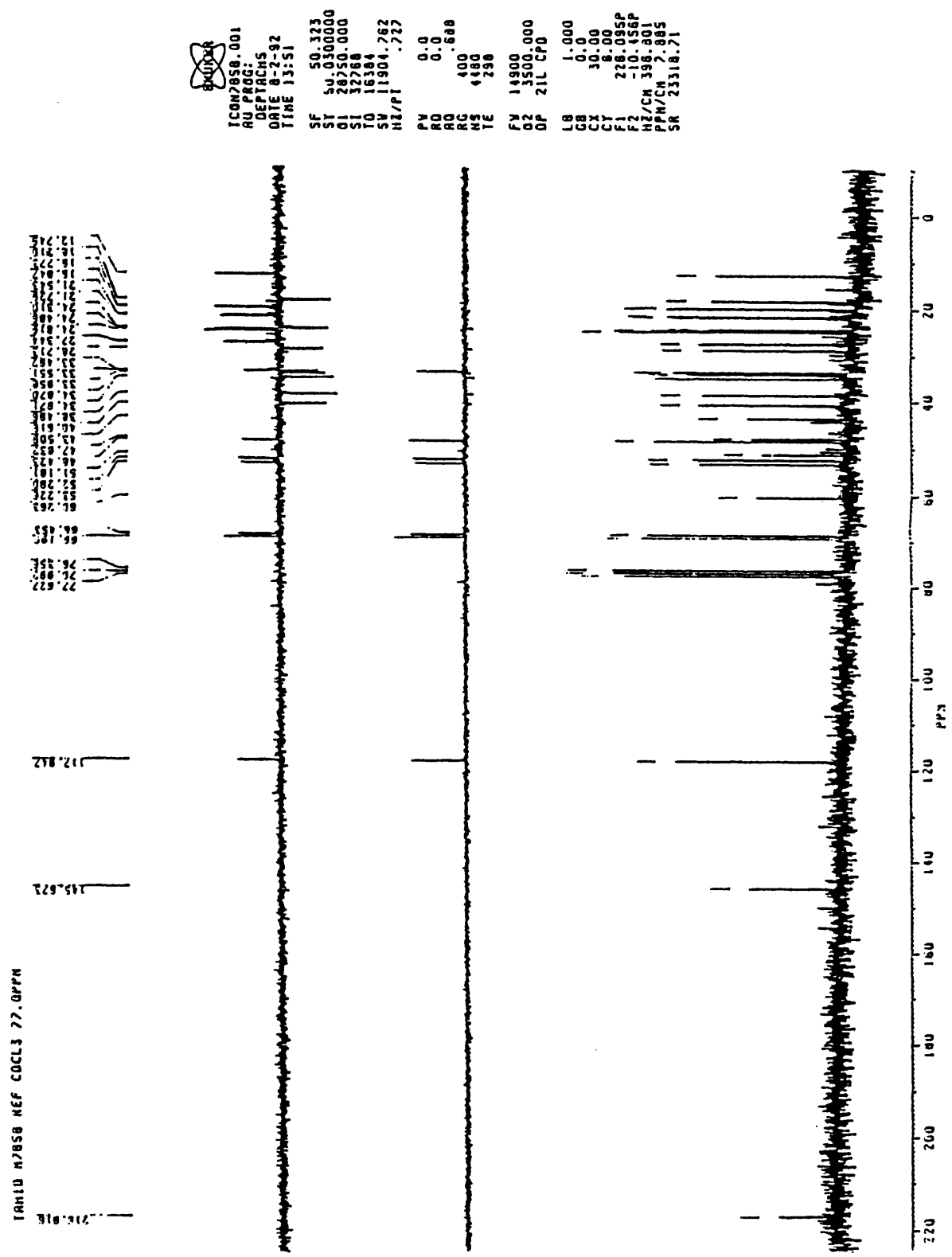

Figure 2. ^{13}C NMR spectrum of compound (3) in $CDCl_3$.

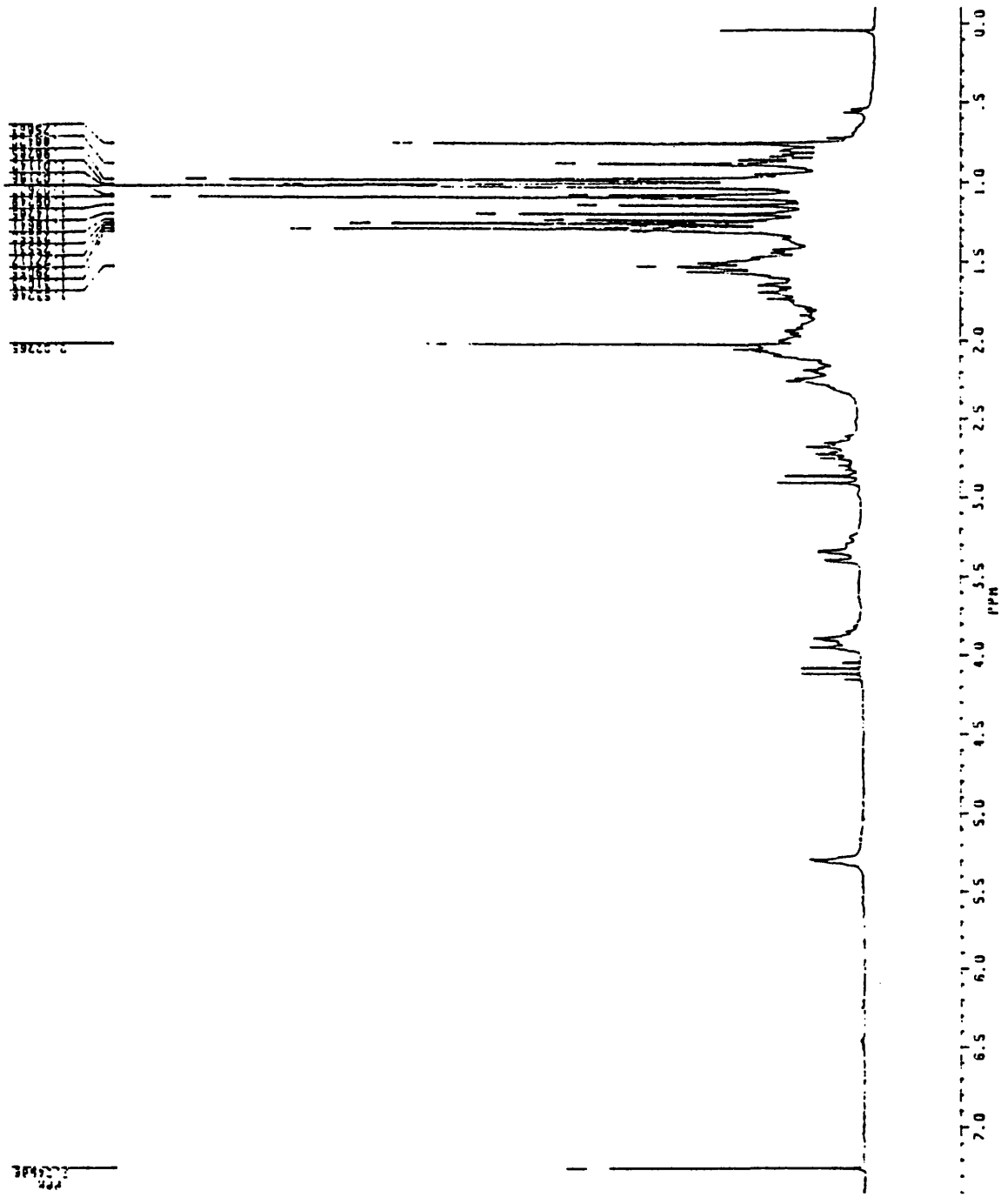

Figure 3. ^1H NMR spectrum of compound (5) in $CDCl_3$.

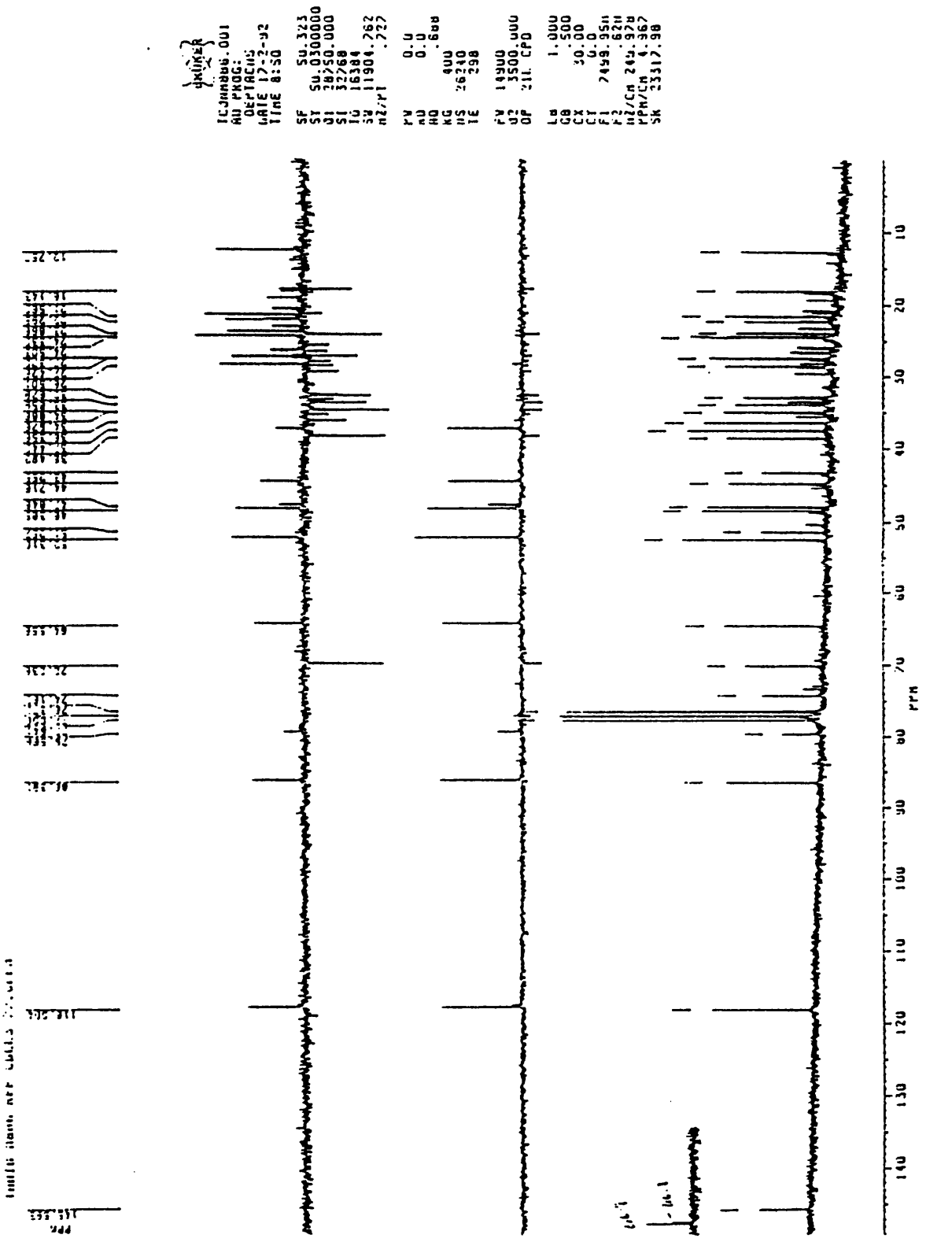

Figure 4. ^{13}C NMR spectrum of compound (5) in $CDCl_3$.

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CHAPTER III

TURRAEA BROWNII

INTRODUCTION

Turraea (Meliaceae) is a rather variable genus, containing some 60-70 species of shrubs and trees, and is widespread in Africa and the islands of the Indian ocean. It is placed in the tribe Turraeeae, subfamily Meliodeae, where it is accompanied by a number of smaller related genera such as *Nymania*.[1] It has recently been reported that *Nymania* was found to contain prieurianin (**1**), a characteristic complex limonoid of the genera *Trichilia* and *Guarea*.[2]

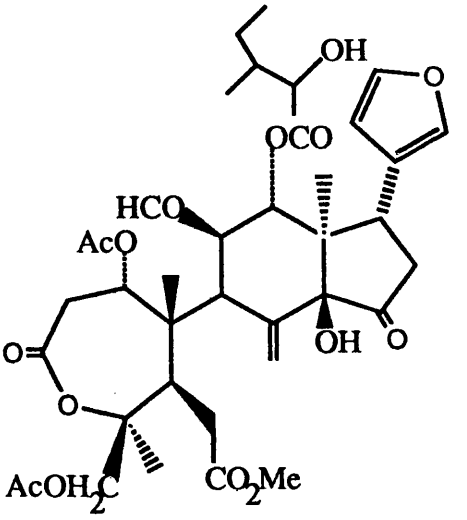
Turraea obtusifolia (Hochstetter) is widely distributed in Southern Africa, although nowhere very common. *Turraea obtusifolia* contains prieurianin, which was identified by comparison of the ^{1}H NMR spectra (at 60°) of the free compound and its acetate. This supports the inclusion of *Trichilia*, *Guarea*, *Nymania* and *Turraea* in the same subfamily.

Turraea floribunda (Hochstetter) is distributed throughout East Africa, being locally rather common. It has a white flower with a powerful scent at night, and is commonly known as a "honeysuckle". Extracts of this plant have been found to contains a mixture of limonoids, apparently of the same group as prieurianin but of a lower oxidation state. Three of these have been isolated and their structures determined, mainly by spectroscopic methods. Their structures were found to be similar to havanensin (**2**), but with oxygen substituents on C-11 and C-12.[3]

Study of a third species, *Turraea nilotica*, revealed the presence of a new protolimonoid named niloticin (**3**) together with two closely related compounds, dihydroniloticin (**4**) and the triol (**5**). Unfortunately no limonoids were found. The spectra of dihydroniloticin were similar to those of niloticin, but instead of a ketonic carbonyl, showed an extra secondary alcohol. The third compound contained the elements of a molecule of water more than niloticin, and has lost the epoxide.[4]

Turraea robusta Guerke, is a small tree found in East Africa. In the Zaramo (Tanzania) language, it is called mzikoziko, and used to cure stomach pains as a tea prepared from the roots. Bentley *et al* have reported the isolation and identification of new compound, mzikonone (**6**), as the major limonoid from the root bark of this plant. From the same plant, they have also isolated a new limonoid, mzikonol (**7**) and a new protolimonoid called turranolide (**8**).[5,6]

In this chapter, we report an investigation of the stem bark of *Turraea brownii* collected in Australia. Several sesquiterpenoid derivatives including humulene, a mixture of humulene apoxide and caryophyllene epoxide and other sesquiterpene alcohols and ketones were isolated. Their structures are discussed. A particularly impressive example of computer-assisted structure elucidation is described.



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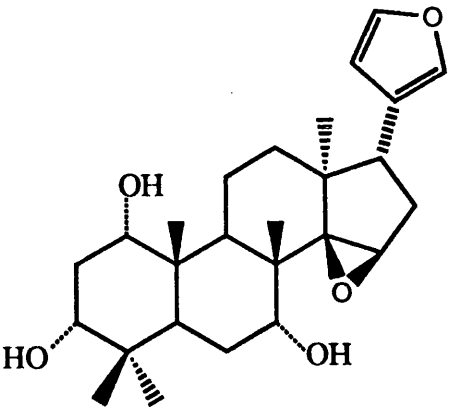

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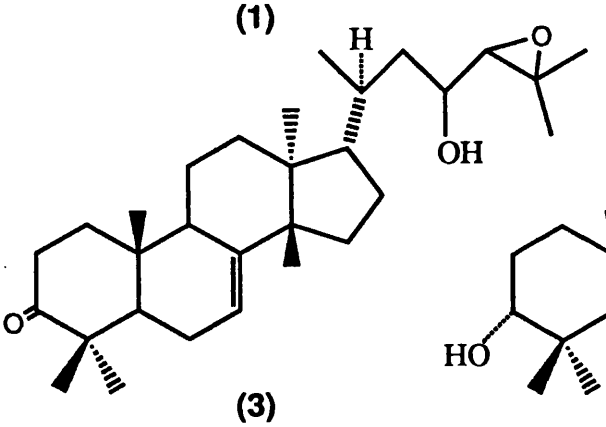

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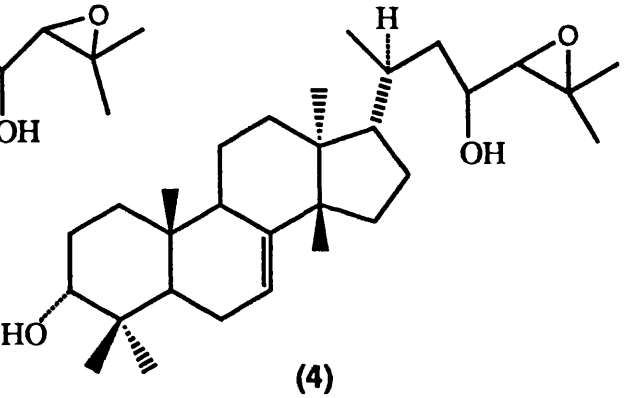

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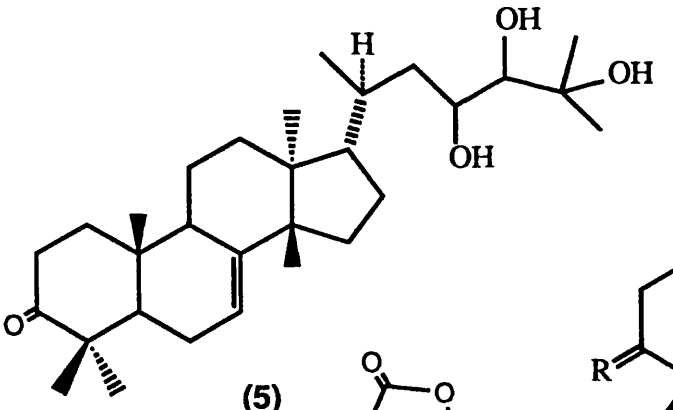

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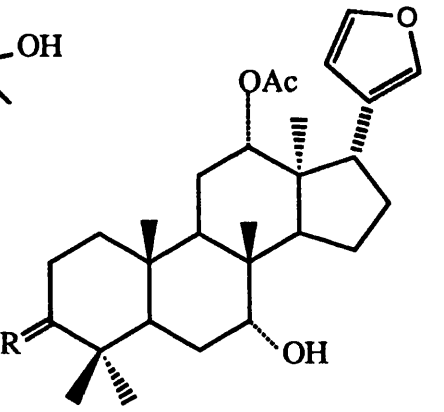
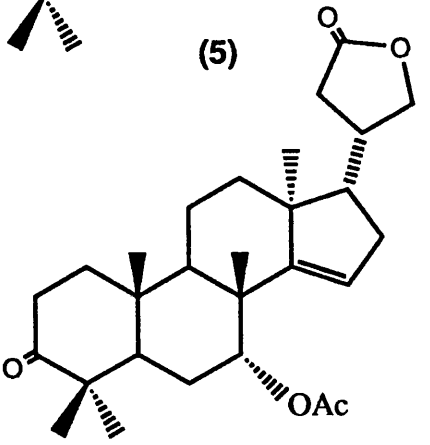


(6) R = O

(7) R = H , β-OH

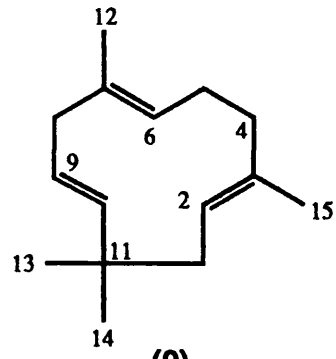


(8)

DISCUSSION

The ethyl acetate extract of the dried ground stem bark of *Turraea brownii* was chromatographed over a dry column of silica gel using a solvent mixture of petroleum ether and ethyl acetate of increasing polarity. Replating of fraction 2 on silica gel TLC preparative plates using petroleum ether afforded humulene (9) as an oily colourless residue. Identification of this compound is based on its NMR spectral data. From its ^{1}H and ^{13}C NMR spectra (Fig.1 and 2), it can be seen there are 24 protons and 15 carbon atoms indicating a sesquiterpene. The ^{1}H NMR spectrum showed the presence of two tertiary methyl groups (6H signal at δ_H 1.08), two vinylic methyl groups (3H doublets centered at δ_H 1.65 and 1.44, J=1.3 Hz), and four olefinic protons located between δ_H 4.9 and 5.6. The pattern of the vinyl protons δ_H 5.61 (dt, J 15.9, 7.4 Hz), 5.17 (dt, J 15.9, 0.8 Hz), and 4.90 (tq, J 7.6, 1.3 Hz) indicated a *trans* disubstituted double bond and two trisubstituted double bonds. The signals for two allylic methylene groups were also apparent at δ_H 2.52 and 1.92 [each 2H (d), J 7.6 Hz)]. These spectroscopic data are in accordance with the published data for humulene[7-10] (see Table 1).

Thus far, the ^{13}C NMR chemical shifts of humulene have not been found in the literature. To assist in the assignment of the ^{13}C shifts of humulene, they were compared with those of humulene epoxide.[11] The effect of the epoxide on C-6 and C-7 is similar to that of a double bond. The signals of four methyl groups C-12, C-13, C-14 and C-15 are located at δ_C 17.9, 26.5, 27.0 and 15.0, respectively. The triplet signal of C-5 is more shielded by the double bond than C-8 and consequently C-5 resonates at higher field [C-5 δ_C 23.3 and C-8 δ_C 40.3]. The doublet signals of C-9 and C-10 are located at δ_C 124.9 and δ_C140.9 since C-9 more shielded by the methyl groups at C-11.



(9)

The product isolated from fraction 10 (F10) by preparative TLC using petroleum ether-ethyl acetate (98-2). gave very interesting NMR spectra. Its ^{1}H NMR spectrum (Fig.3) exhibited seven methyl groups comprising one vinyl methyl (δ_H 1.54) and six quaternary methyls [δ_H 0.96, 0.98, 1.05, 1.08, 1.17 and 1.28 - the chemical shifts of the two quaternary methyls at δ_H 1.17 and 1.28 suggests their being attached to a carbon linked to oxygen[11]], a vinyl group system at δ_H 5.1-5.4, an exocyclic methylene at 4.86 and 4.97, and two one proton signals centred at δ_H 2.87 (dd, J 8 Hz) and 2.52 (dd, J 8.4 Hz) arising from trisubstituted epoxides. Its ^{13}C DEPT NMR spectrum (Fig.4) showed that there are 30 carbon atoms consisting of 7 methyls, 10 methylenes, 7 methines and 6 non protonated carbons. The assignment of the functionality can be seen in Table 2, as follow;

Table 2. ^{13}C NMR assignment of compound F10B4.

Chemical shifts (ppm)	Functionality
15.0, 16.9, 17.1, 21.6, 27.2	-methyl groups
59.8, 61.9, 63.2, 63.7	-epoxides
112.7, 122.0, 125.7, 131.8, 143.0, 151.7	-double bonds

The highest mass peak was m/z 331 but there was no good fragmentation pattern.

It was difficult to relate the above data to known triterpenoid systems and so we turned to 2D NMR. Proton-proton COSY and direct $^{13}C/^{1}H$ two dimensional chemical shift correlation (HMQC) experiments were run (see Figures 5 - 7) and led to the assigment of some part structures as shown.

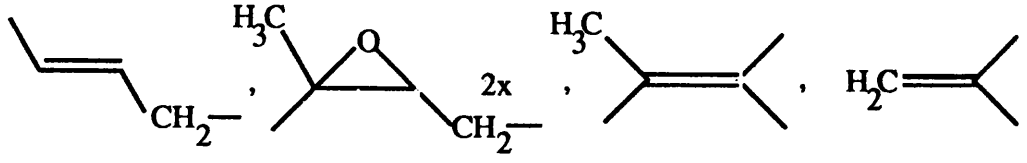
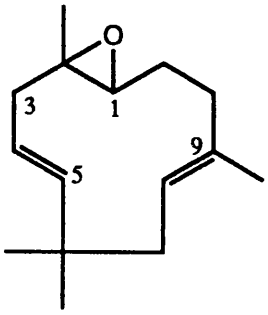
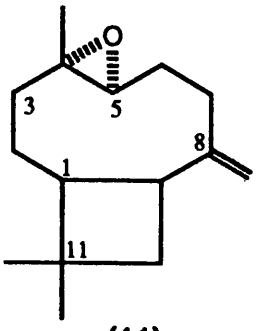


Figure 1. Part structures of F10B4

Clearly more connectivity was required and so a long-range $^{13}C/^{1}H$ experiment (HMBC) was run using inverse detection which greatly increases the sensitivity of the experiment (see Figures 11-12). Many correlations were observed, so many that it was very difficult to assimilate the data and use them for derivation of a rational structure. In recent years Dr. J. M. Nuzillard of the University of Reims has developed a computer programme which is capable of using data for HMQC and HMBC (whether $^{2}J_{CH}$ or $^{3}J_{CH}$) spectra to assign structures automatically.[14] It was decided to use this programme for F10B4. Since there was some overlap in the ^{1}H NMR spectra only clearly defined correlations in the HMQC and HMBC spectra were used (see Fig. 5 and 7). Much to our surprise the computer produced a solution consisting of humulene epoxide (**10**) and caryophyllene epoxide (**11**). Comparison with the ^{13}C chemical shifts of humulene epoxide (**10**) and caryophyllene epoxide (**11**) (Table 3) confirmed that F10B4 was indeed a mixture of the two compounds. GCMS analysis revealed the components of the mixture with retention times of 14.45 and 15.02 minutes respectively.



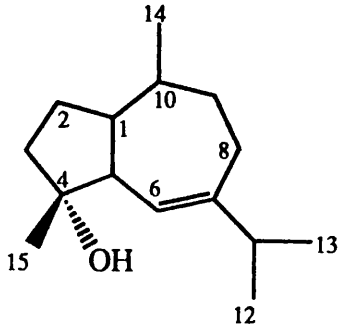
(10)



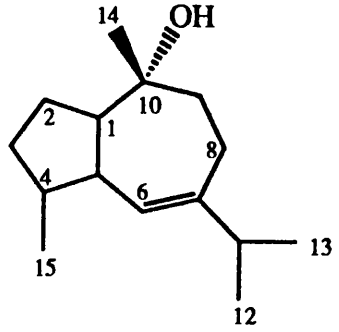
(11)

This represents a particularly impressive example of the power of computer assisted structural elucidation. The computer lacks the prejudices of a human and remains uninfluenced by biogenetic considerations. It does not require to know whether long range correlations between carbons and protons are $^{2}J_{CH}$ or $^{3}J_{CH}$ since the programme considers all possibilities. There is little doubt that this computer approach to structural elucidation will find wide application once the advantages and the possibilities are realised.

Preparative TLC of fraction 11 (F11) with petroleum ether-ethyl acetate (95-5) as eluent gave compound F11B3. Its ^{1}H and ^{13}C NMR spectra (Fig.7 and 8) indicated that it was a sesquiterpenoid, $C_{15}H_{26}O$. The ^{1}H NMR spectrum shows the presence of an isopropyl grouping [δ_H 0.86 and 0.95 (both δ, J 6.5 Hz)], and a quaternary methyl [δ_H 1.17 (s)] which seems to be attached to a carbon carrying a tertiary hydroxyl group (δ_C 73.5). A signal at δ_H 5.48 indicated a vinyl proton which is associated with trisubstituted double bond whose ^{13}C resonances appear at δ_C 148.8 (s) and 124.1 (d). The molecule is therefore bicarbocyclic. Comparison with literature data[10], suggested that F11B3 was a guaiane possibly 6-guaien-4-ol (**12**) [Table 4]. The data do not exclude 6-guaien-10-ol (**13**) as a possible structure. No reference to (**13**) was found in the literature.



(12)



(13)

Table 4. ^1H NMR chemical shifts of F11B3 and 6-guaien-4-ol in $CDCl_3$[10].

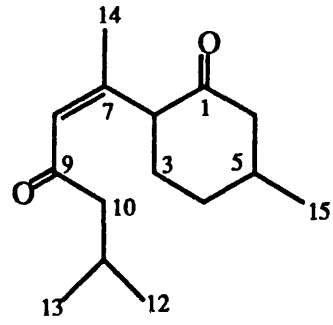
Proton	Experimental	6-Guaien-4-ol (12)
6-H	5.48 d	5.51 d
12-H	0.96 d	0.98 d
13-H	0.95 d	0.97 d
14-H	0.86 d	0.89 d
15-H	1.17 s	1.20 s

In addition to the signals for the trisubstituted double bond and the tertiary carbinol referred to above, the DEPT spectrum of F11B3 showed resonances for four methyl groups (δ_C 15.5, 21.2, 21.4 and 30.4), four methylenes (δ_C 23.9, 24.5, 32.7 and 40.9) and four methines (δ_C 36.8, 37.5, 41.7 and 49.7) consistent with a 6-guaienol structure.

Preparative TLC of fraction 16 (F16) using first petroleum ether-ethyl acetate (90-10) and twice petroleum ether-ethyl acetate (80-20) afforded compound F16B2 which was readily identified on a plate because of its UV activity. Compound F16B2 was not very stable and was obtained only in limited amount. From the ^1H and ^{13}C NMR data discussed below it seemed likely that F16B2 was a bisabolene sesquiterpenoid. 7-Bisabolene-1,9-dione (**14**) is suggested as a possible structure. However definitive proof is lacking.

The ^1H NMR spectrum (Fig.14), reveals three secondary methyl groups [δ_H 0.76, 1.02 and 1.04 (3xd, J 6.5, 6.7 and 6.8 Hz)], one vinyl methyl group [δ_H 2.10 (s)] and one olefinic proton [δ_H 5.86 (bs)]. The inequality of the two methyl groups at 12 and 13 is due to the chirality of the molecule. The chemical shift of the vinyl proton suggests it is situated α to the carbonyl group of a conjugated enone. The vinyl methyl must be attached to the β position. Its deshielded nature requires that it should be near the other

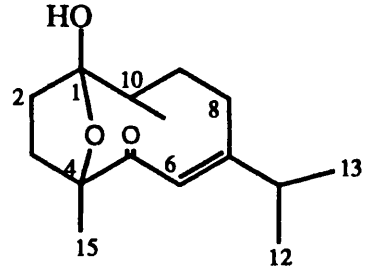
carbonyl group. Structure (**14**), 7-bisabolene-1, 9-dione, accomodates this requirement.



(14)

The ^{13}C NMR spectrum (Fig.15) confirmed the presence of the trisubstituted double bond [δ_C 127.9 (d, C-8) and 170.4 (s, C-7)], two ketonic carbonyl carbons, one conjugated [δ_C 203.5, C-1 and 209.1, C-9] and four methyl carbons [δ_C 16.2, 22.6, 20.9 and 33.7, C-14, C-12, C-13 and C-15].

A second UV active, unstable sesquiterpenoid F17B3 was obtained, again in minor amount, from fraction 17 (F17) by preparative TLC using petroleum ether-ethyl acetate (80-20) (x3). Structure (**15**), 1,4-epoxy-1-hydroxy-6-germacren-5-one, is suggested for this compound on the following basis.



(15)

Its ^1H NMR spectrum (Fig.16) shows resonances for three overlapping methyl groups (at δ_H 0.95), one tertiary methyl group (δ_H 1.51), and olefinic proton [δ_H 6.09 (s)]. The ^{13}C NMR spectrum (Fig.17) confirmed the presence of a trisubstituted double bond [δ_C 123.6 (d) and 150.5 (s)], and additionally revealed two oxygenated carbons, one an acetal [δ_C 83.1 (s) and 91.9 (s)],

four methyl groups [δ_C 16.3, 17.0, 17.8 and 25.9] and a weak ketonic carbonyl resonance [δ_C 209.8]. The molecular formula $C_{15}H_{24}O_3$ was deduced from the DEPT spectrum. There is no definitive evidence for the structure of F17B3 but the functionality can be rationally assembled as in (**15**). Structure (**15**), 1,4-epoxy-1-hydroxy-6-germacren-5-one, accomodates the deshielded nature of vinyl proton and its lack significant coupling, the deshielded nature of the tertiary methyl group which is presumably affected by the adjacent ketonic carbonyl and the lack of equilibration of the ketal (acetal) since it can only be formed by addition from one face of the original carbonyl group.

The above sesquiterpenoids clearly require further evidence to confirm their structures. It is unfortunate that a combination of their instability and lack of time and material prevented further work.

EXPERIMENTAL

The dried ground stem bark (426.37 g) was extracted successively by exhaustive percolation with ethyl acetate and methanol. Evaporation of the ethyl acetate extract under pressure, left a dark green oily residue (16.13 g) which was extracted with ether. Evaporation of the ether afforded a dark green residue (11.93 g) which was subjected to chromatography on silica gel dry column (100 g). Elution was carried out using mixtures of petroleum ether (40^{o}-60^{o}), ethyl acetate and methanol of increasing polarity. A total of 38 fractions (50 ml each) was collected. The fractions were combined into groups according to the similarity of their TLC patterns on silica gel GF_{254} using mixtures of petroleum ether and ethyl acetate.

Replating of fraction 2 (F2) on silica gel preparative plates (1 mm thickness) with petroleum ether afforded humulene (43.01 mg) as a colorless oil, which was identified by comparison its ^{1}H chemical shift data with literature values.[7]

Replating of fraction 10 (F10) on preparative plates using petroleum ether-ethyl acetate (98-2) as the solvent gave a strongly UV active band (F10B4) as the major component. This band was removed and extracted with ethyl acetate to yield F10B4 as a colorless residue (15.4 mg). Analysis of the spectrometric properties of F10B4 led eventually to the conclusion (see text) that it was a mixture of humulene epoxide and caryophyllene epoxide. GC-MS analysis on CP Sil 5 CB capillary column gave two peaks with retention times 14.45 min (m/z 220) and 15.02 min (m/z 220) consistent with the presence of humulene epoxide (**10**) and caryophyllene epoxide (**11**).

Fraction 11 (F11) was chromatographed on silica gel preparative plates using double elution with petroleum ether-ethyl acetate (95-5) as the solvent. Extraction of band 3 evaporation of the solvent afforded an unstable oily residue of F11B3 (12.5 mg), the major component. This

compound was tentatively identified as 6-guaien-10-ol (**13**) on the basis of its spectroscopic properties.

Preparative TLC of fraction 16 (F16) using first petroleum ether-ethyl acetate (90-10) and then twice with petroleum ether-ethyl acetate (80-20) resulted six bands. Band 2, which was UV active (254 nm), was extracted with ethyl acetate to give the unstable compound F16B2 (23.3 mg). The structure 7-bisabolone-1, 9-dione (14) is proposed for F16B2 on the basis of its spectroscopic properties. HMBC and HMQC experiments were run but due to the small quantity and the unstable nature of the compound it proved impossible to intepret the results.

Fraction 17 (F17) was subjected to preparative TLC using first petroleum ether-ethyl acetate (85-15) and then twice with petroleum ether-ethyl acetate (80-20). Several bands were observed. Band 3 was removed and extracted with ethyl acetate. Evaporation of the solvent yielded compound F17B3 (17 mg) as an unstable oil. The structure of the compound is suggested to be 1, 4-epoxy-1-hydroxy-6-germacren-5-one (**15**).

Table 1. ^{1}H and ^{13}C NMR chemical shifts of F2 and humulene[5,7] in $CDCl_3$.

Carbon	Experimental			References	
	δ_C (mult)		δ_H (mult, J in Hz)	δ_H (mult, J in Hz)	δ_H
1	41.9	t	1.91 (d, 7.5)	1.91 (d, 7.6)	
2	125.8	d	4.90 (t, 7.6)	4.92 (t, 7.6)	4.92
3	132.9	s	-	-	-
4	39.7	t	1.9 - 2.1 (m)	2.07 - 2.11 (m)	1.82-2.23
5	23.3	t			
6	127.7	d	4.97 (bt, ca 6)	4.92	
7	139.0	s	-	-	-
8	40.3	t	2.52 (d, 7.3)	2.51 (d, 7.3)	2.48
9	124.9	d	5.62 (dt, 7.4,15.9)	5.61 (dt, 7.3,16)	5.64
10	140.9	d	5.15 (d, 15.87)	5.14 (d, 16)	5.18
11	37.3	s	-	-	-
12	17.9	q	1.65 (s)	1.64 (s)	1.63
13	26.5	q	1.08 (s)	1.06 (s)	1.06
14	27.0	q	1.08 (s)	1.06 (s)	1.06
15	15.0	q	1.44 (d, 1.3)	1.43 (d, 2)	1.43

Notes: s = singlet
d = doublet
t = triplet
q = quartet
b = broad
mult = multiplicity

Table 3. ^{13}C NMR chemical shifts of humulene epoxide and caryophyllene epoxide in $CDCl_3$.

Carbon	Humulene epoxide				Caryophyllene epoxide			
	δ_C	(mult)E*	δ_C	(mult)R*	δ_C	(mult)E*	δ_C	(mult)R*
C - 1	61.87	d	61.87	d	50.68	d	50.95	d
C - 2	63.17	s	63.11	s	30.15	t	30.29	t
C - 3	42.53	t	42.70	t	39.10	t	39.32	t
C - 4	122.04	d	122.25	d	59.76	s	59.67	s
C - 5	143.05	d	143.12	d	63.69	d	63.69	d
C - 6	36.21	s	36.53	s	29.51	t	29.96	t
C - 7	40.53	t	40.36	t	29.73	t	30.09	t
C - 8	125.66	d	125.83	d	151.76	s	151.89	s
C - 9	131.84	s	131.94	s	48.68	d	48.87	d
C - 10	36.57	t	36.78	t	39.70	t	39.91	t
C - 11	24.69	t	24.89	t	33.96	s	34.06	s
C - 12	17.15	q	17.29	q	16.95	q	17.03	q
C - 13	25.48	q	25.67	q	27.15	q	27.70	q
C - 14	28.97	q	29.05	q	21.58	q	21.90	q
C - 15	15.03	q	15.01	q	112.70	t	112.83	t

E* - Experimental.
R* - Reference[9].

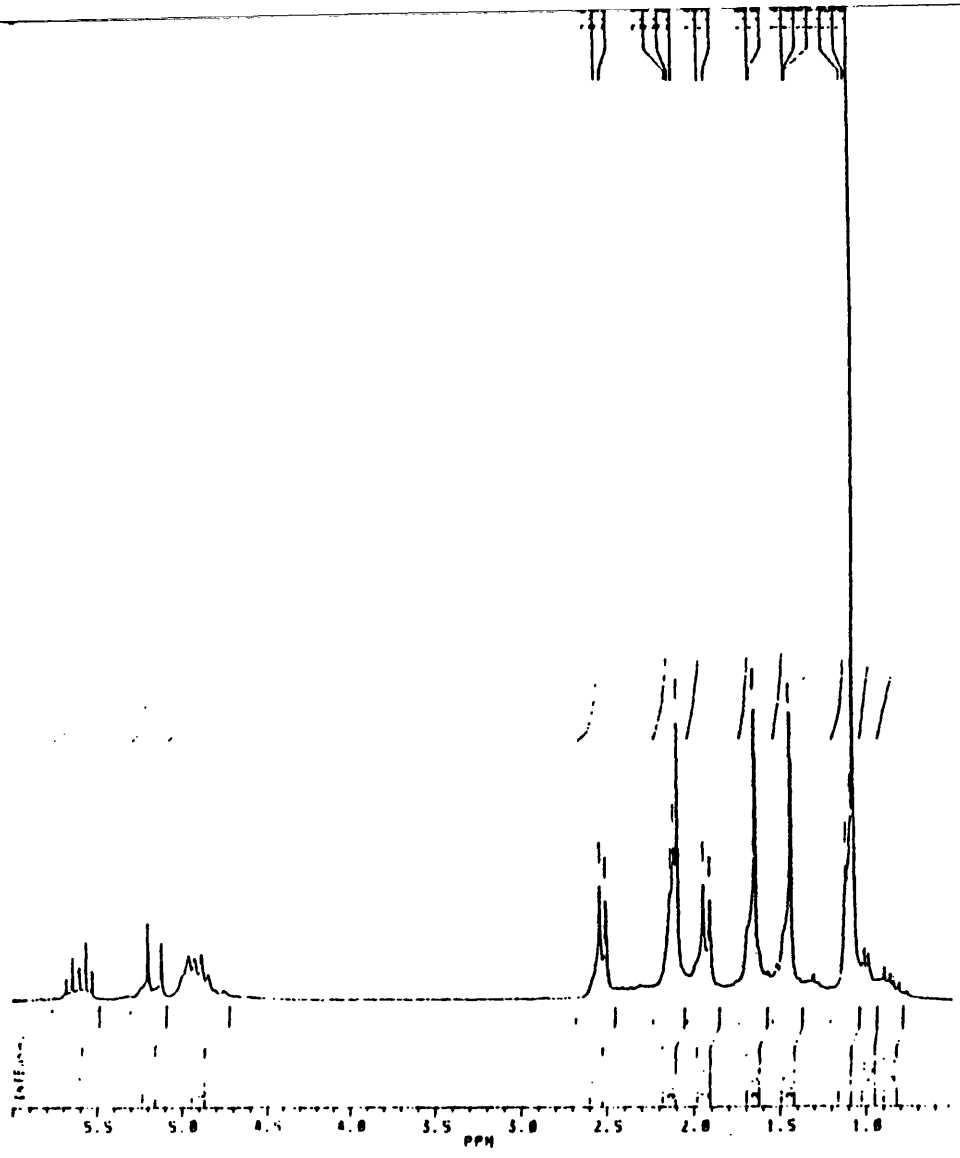


Figure 1. ^{1}H NMR spectrum of humulene (F2) in $CDCl_3$.

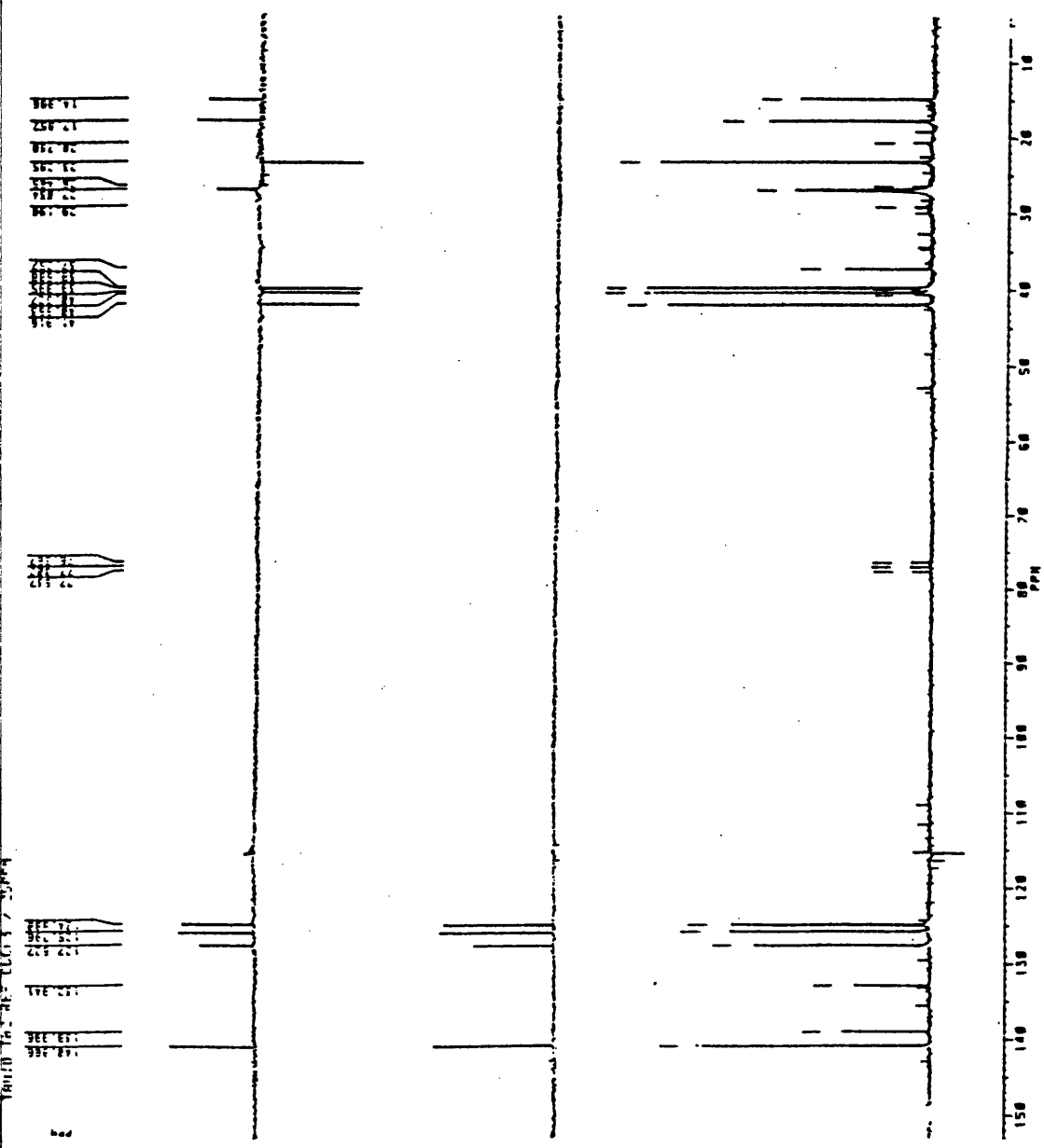


Figure 2. ^{13}C NMR spectrum of humulene (F2) in $CDCl_3$.

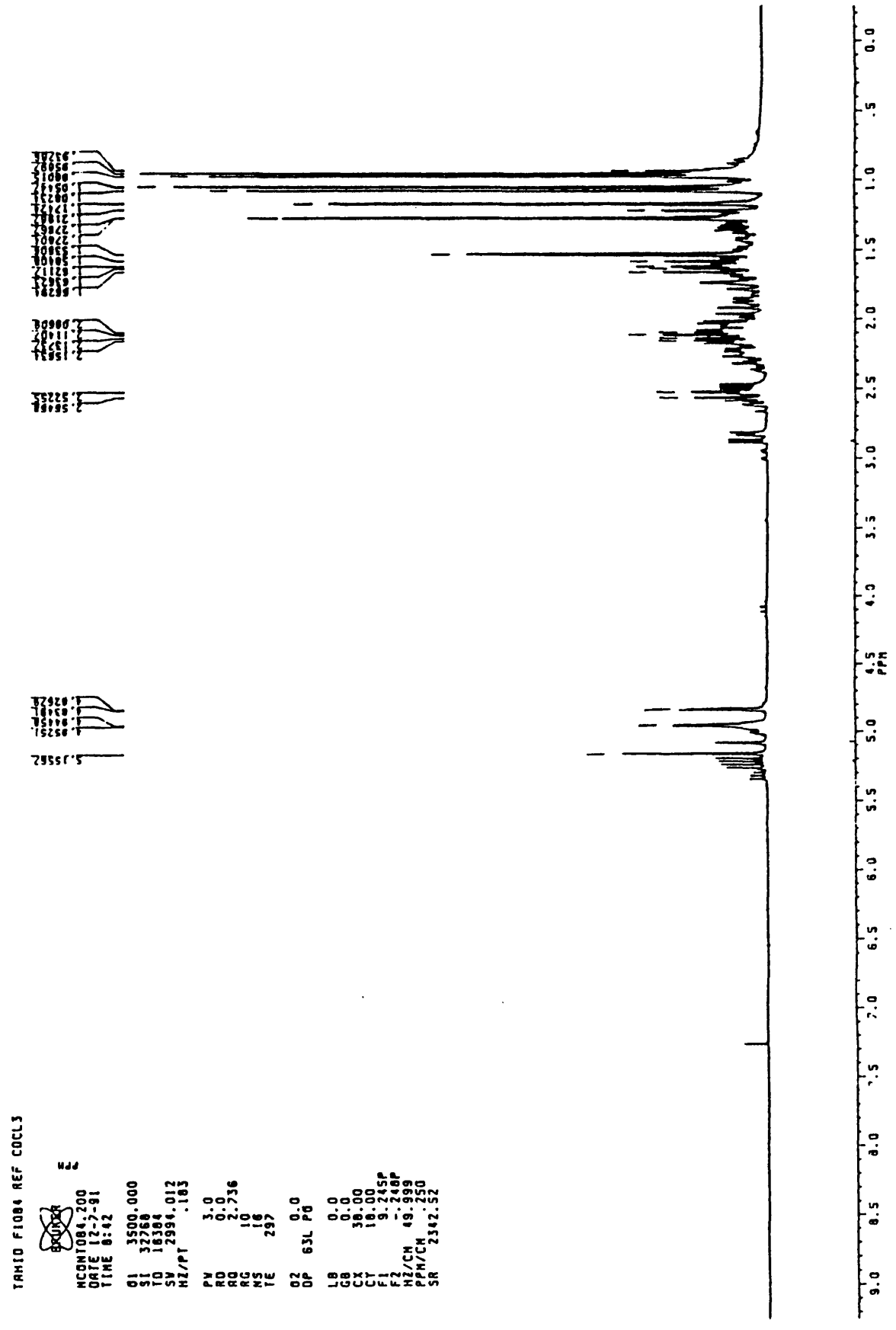


Figure 3. ^1H NMR spectrum of F1084 in $CDCl_3$.

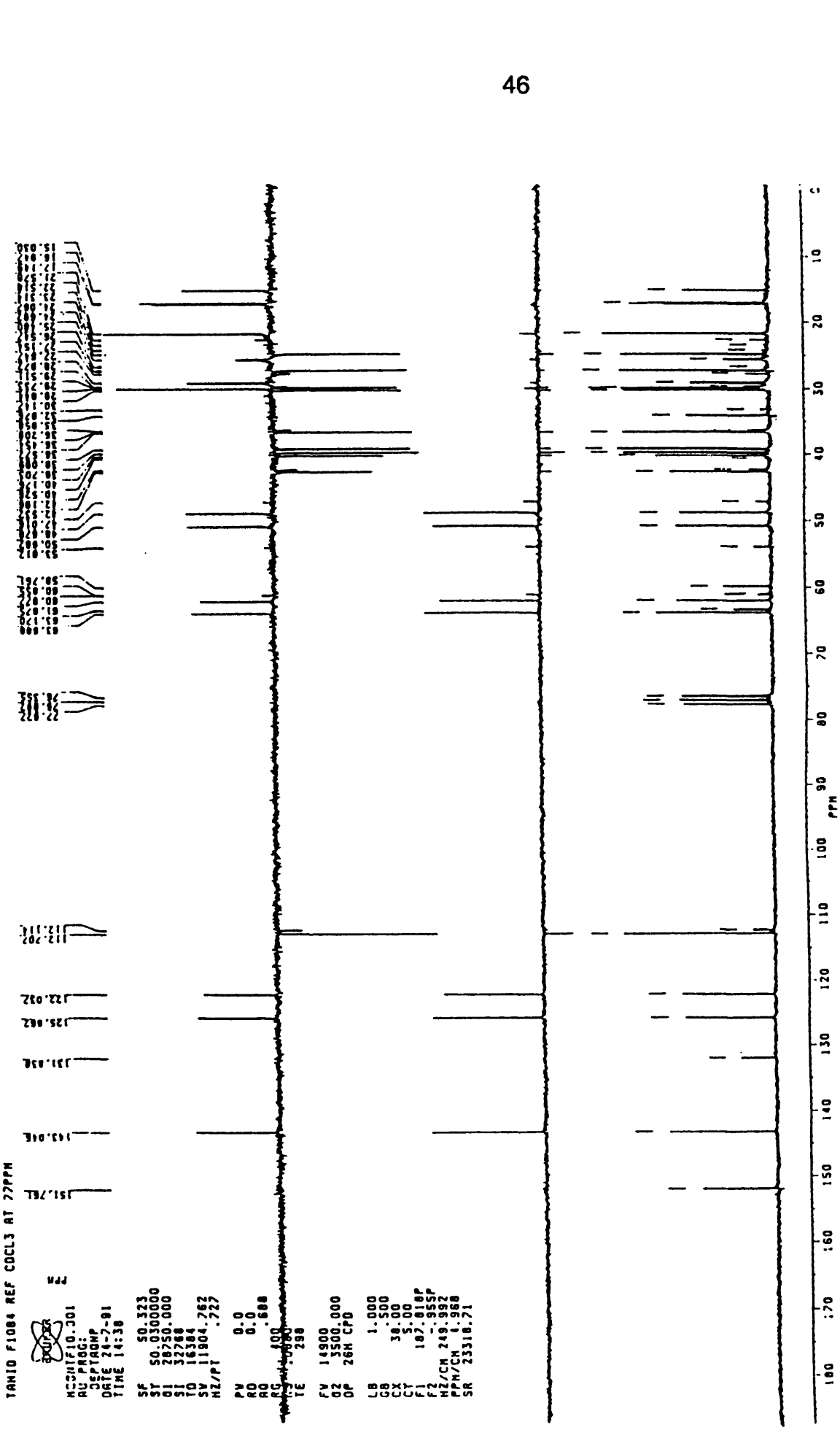


Figure 4. ^{13}C DEPT NMR spectrum of F1084 in $CDCl_3$.

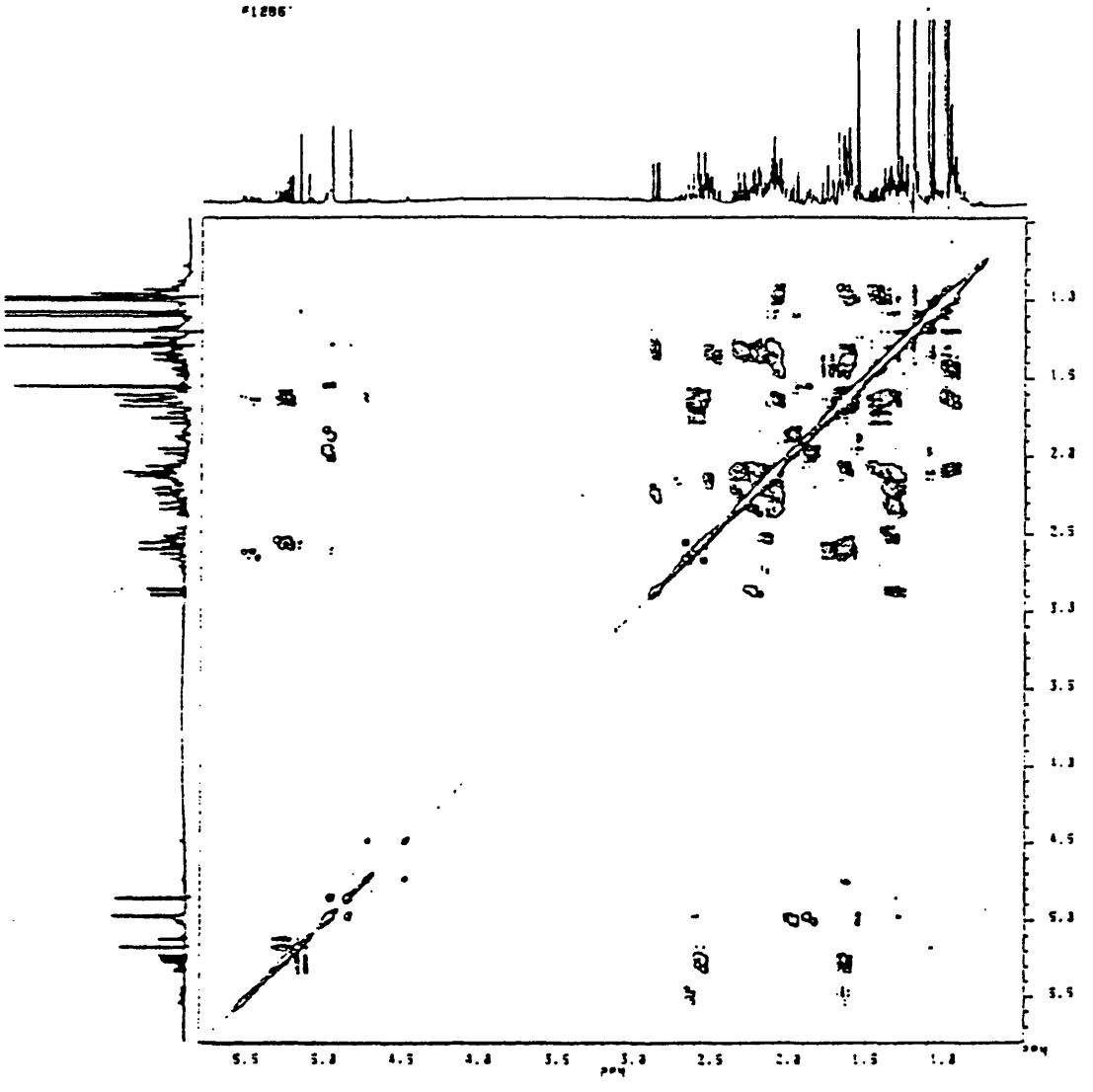


Figure 5. Contour plot of a COSY 90 experiment with F110B4 in $CDCl_3$.

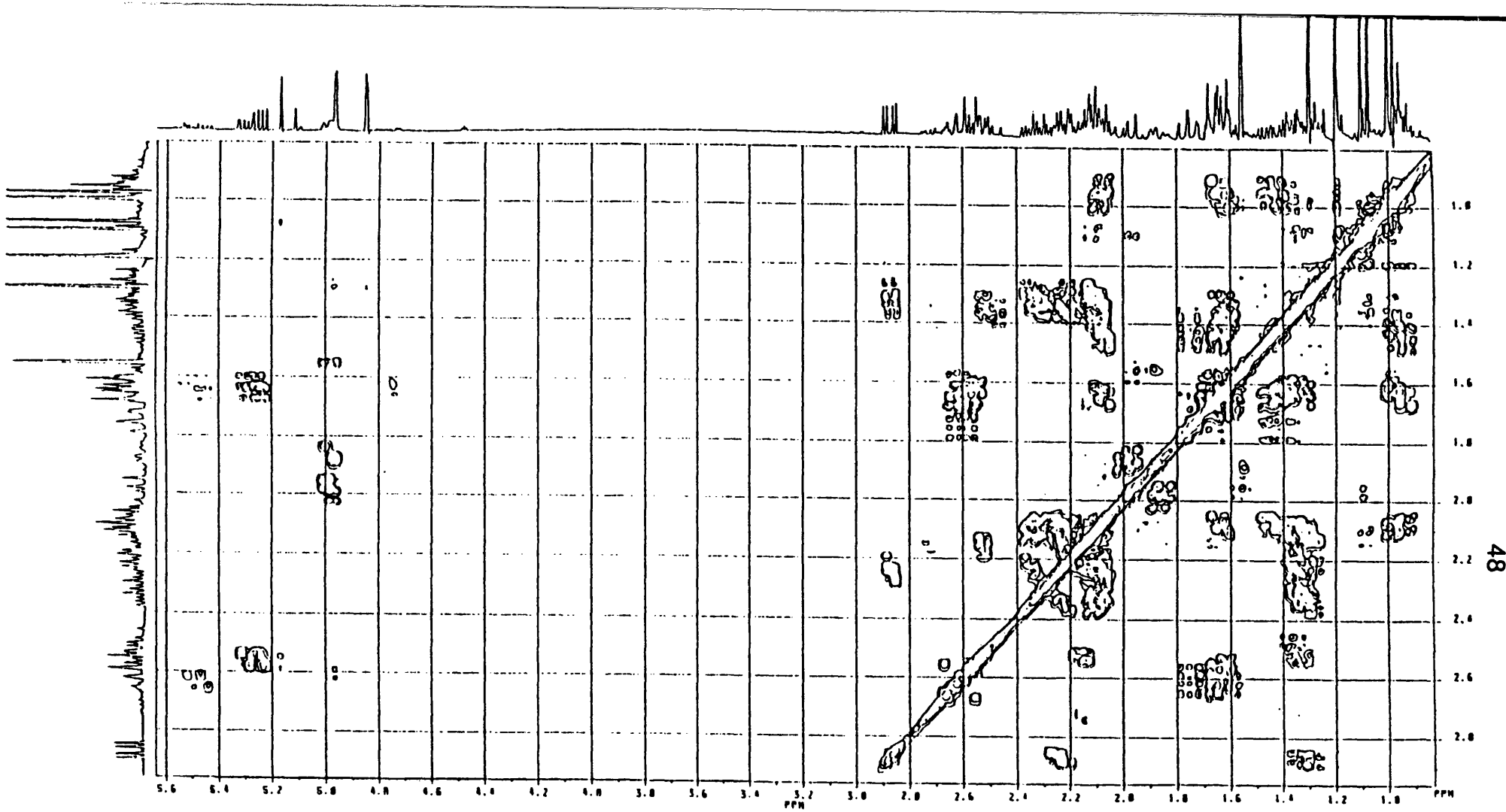


Figure 6. Contour plot of ^{1}H-^{1}H COSY spectrum of F10B4 in $CDCl_3$. -Expansion.

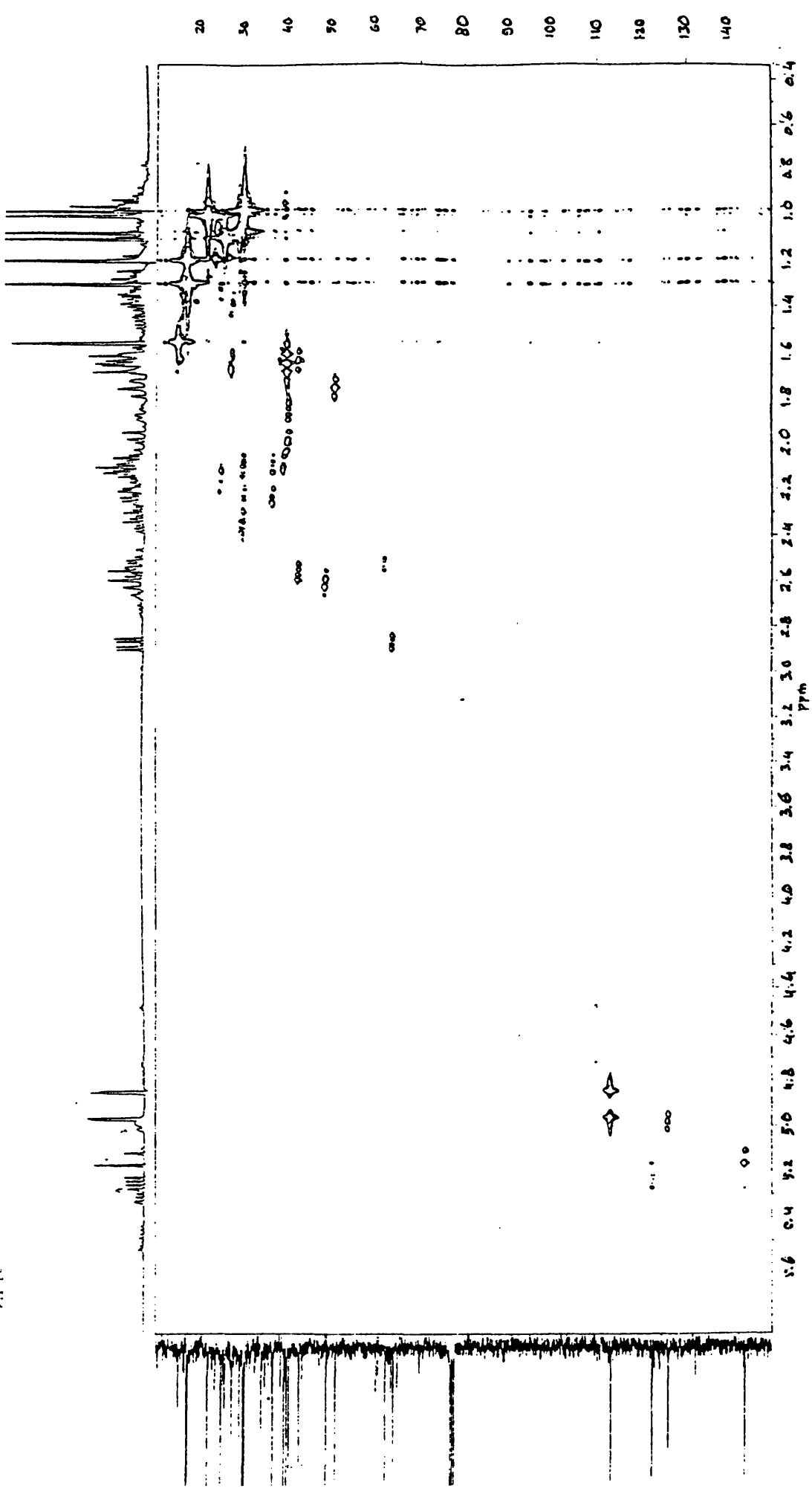


Figure 7. Contour plot of a $^{13}C/^{1}H$ 2D chemical shift correlation of F10B4 in $CDCl_3$.

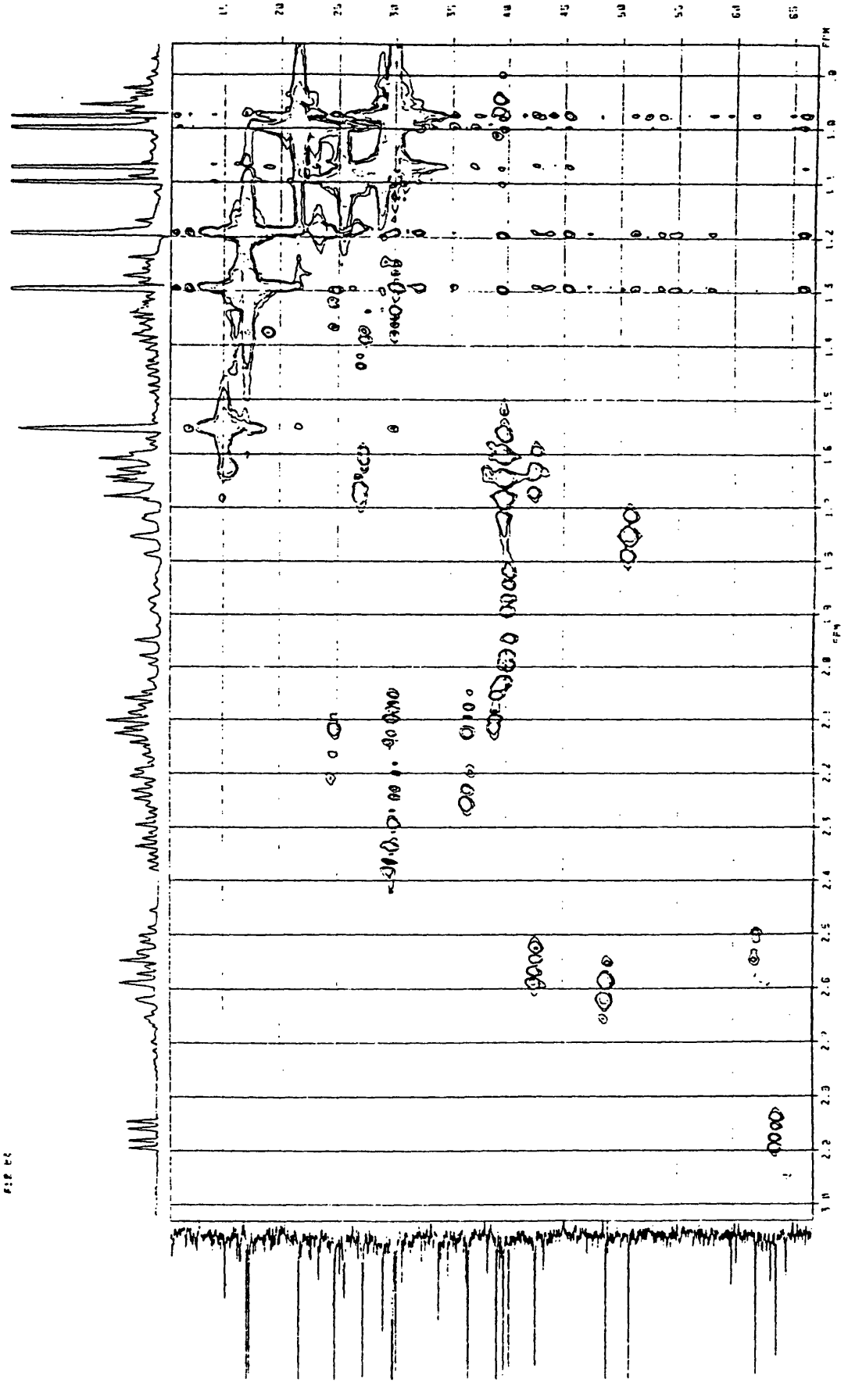


Figure 8. Direct $^{13}C/^{1}H$ 2D chemical shift correlation of F10B4 in $CDCl_3$- Expansion.

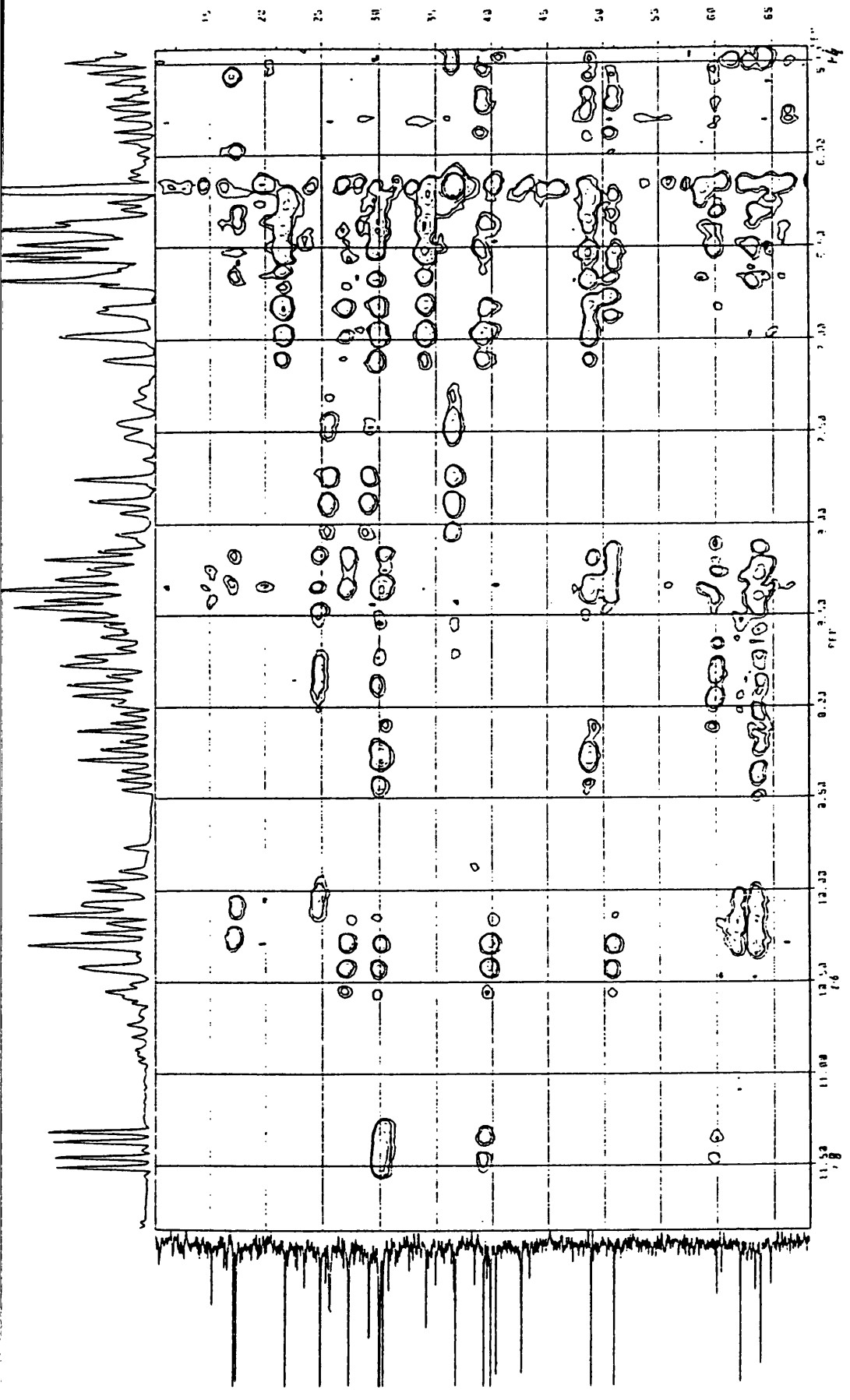


Figure 9. Part of long-range $^{13}C/^{1}H$ 2D correlation of F10B4 in $CDCl_3$.

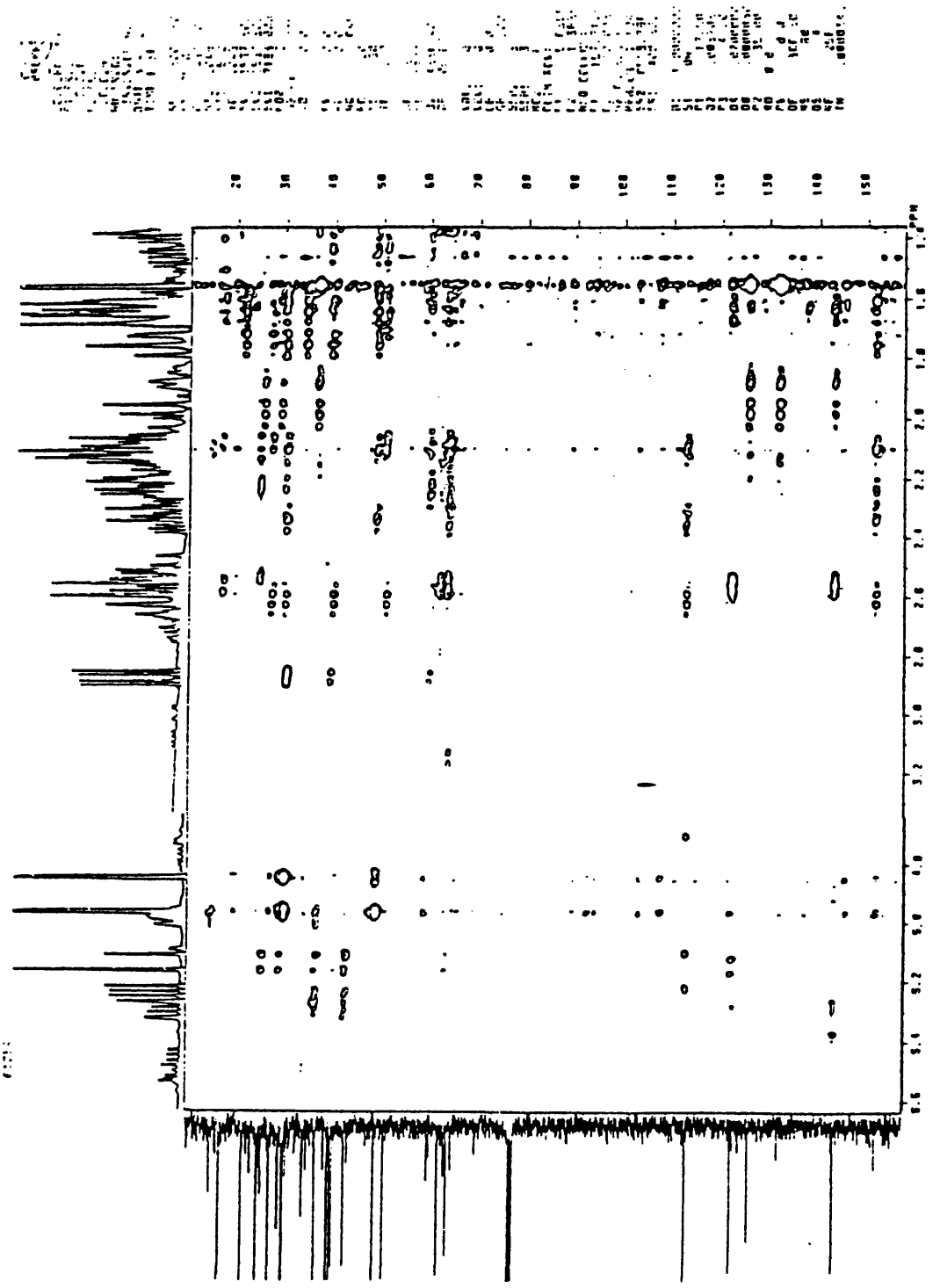


Figure 10. Long-range $^{13}C/^{1}H$ 2D correlation of F1084 in $CDCl_3$.

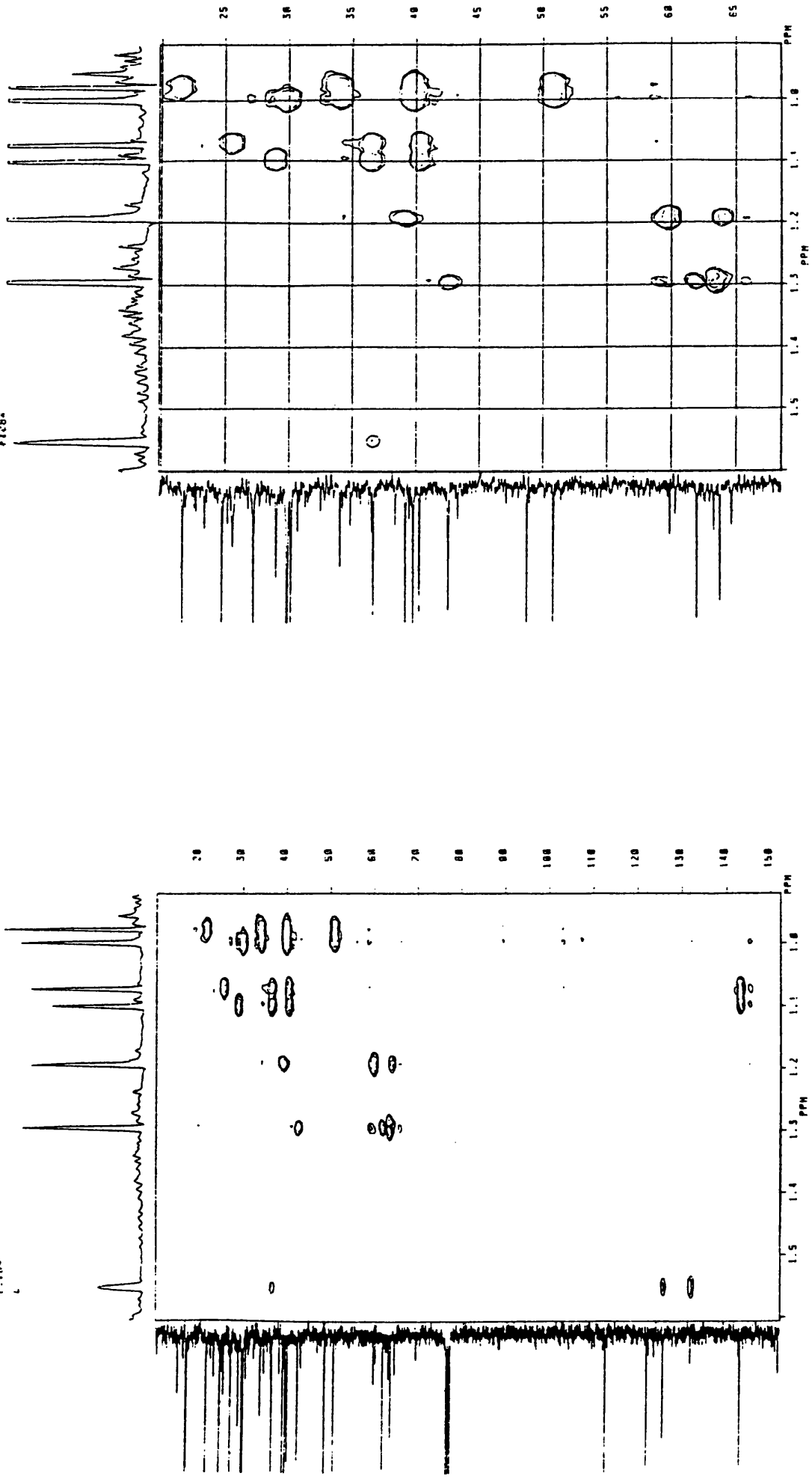


Figure 11. Part of long-range $^{13}C/^{1}H$ 2D correlation of F10B4 in $CDCl_3$.

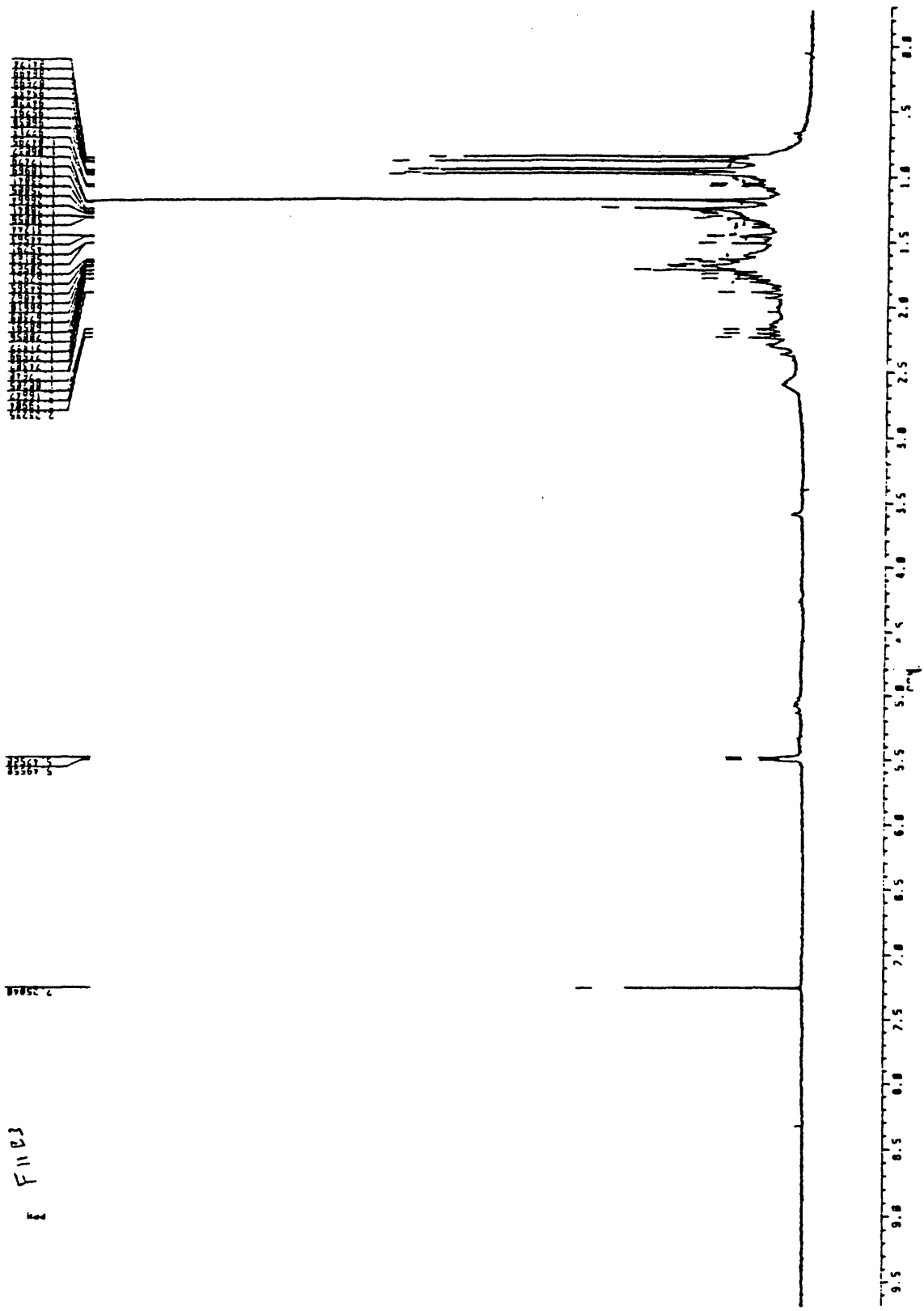


Figure 12. ^1H NMR spectrum of F11B3 in $CDCl_3$.

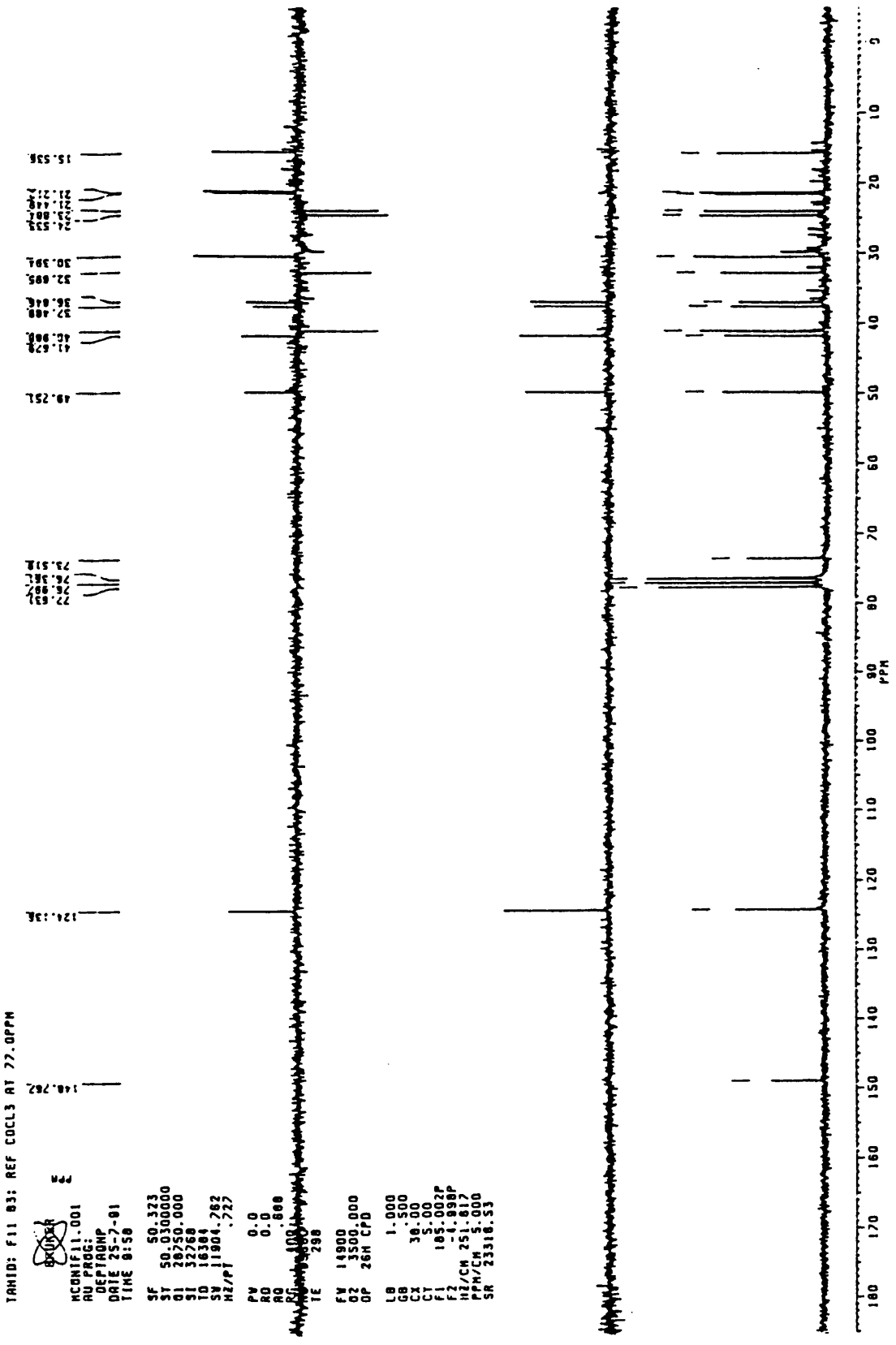


Figure 13. ^{13}C NMR spectrum of F11B3 in $CDCl_3$.

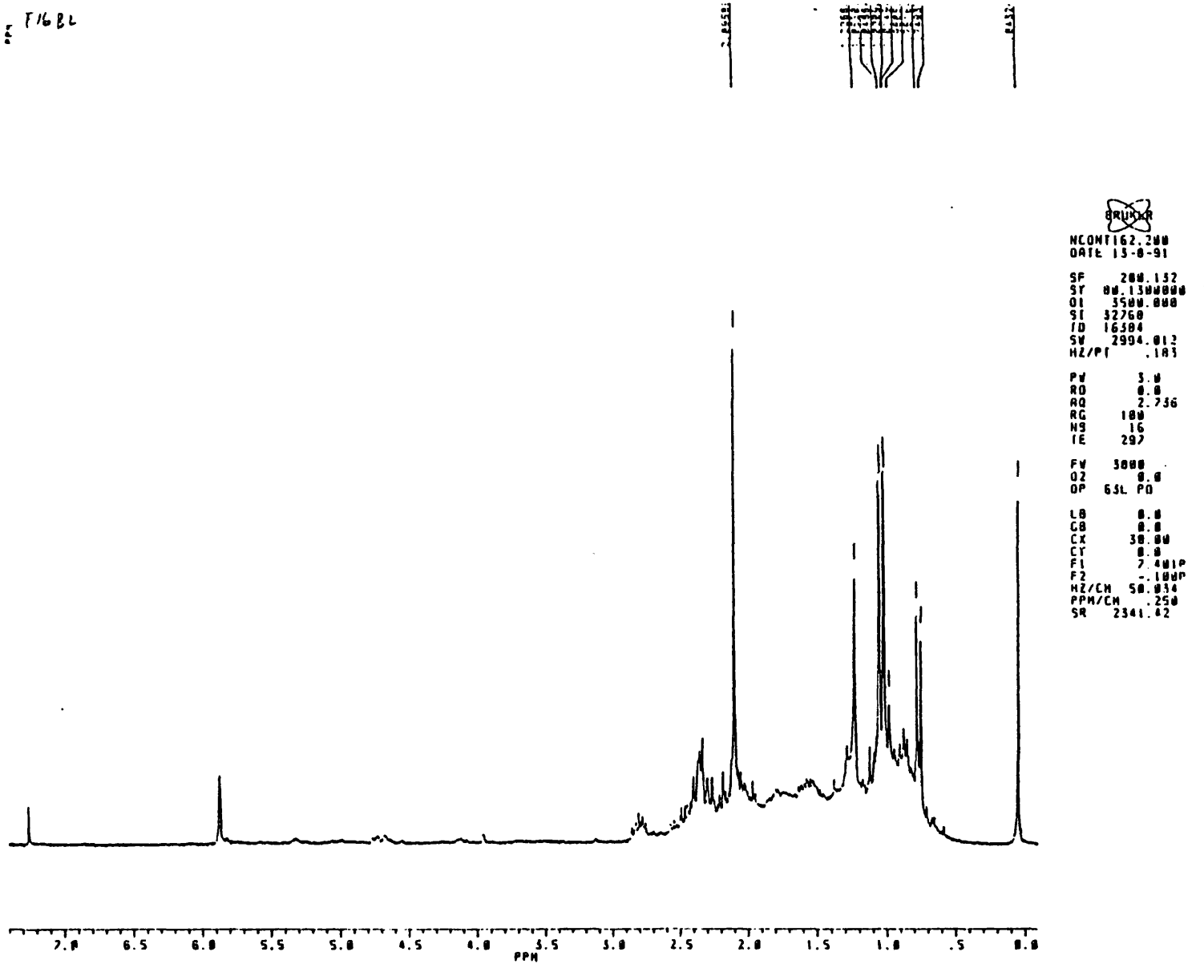


Figure 14. ^{1}H NMR spectrum of F16B2 in $CDCl_3$.

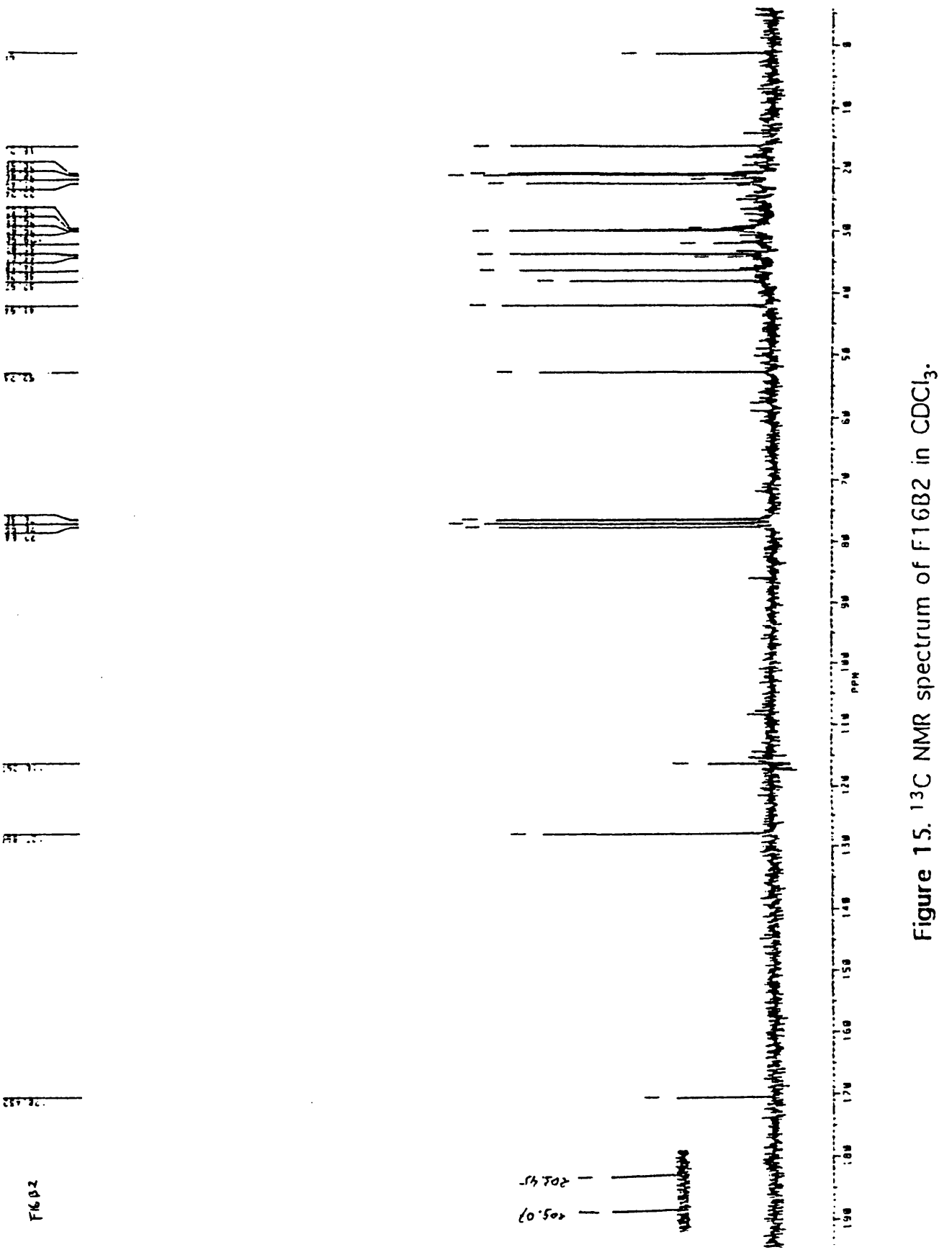


Figure 15. ^{13}C NMR spectrum of F16B2 in $CDCl_3$.

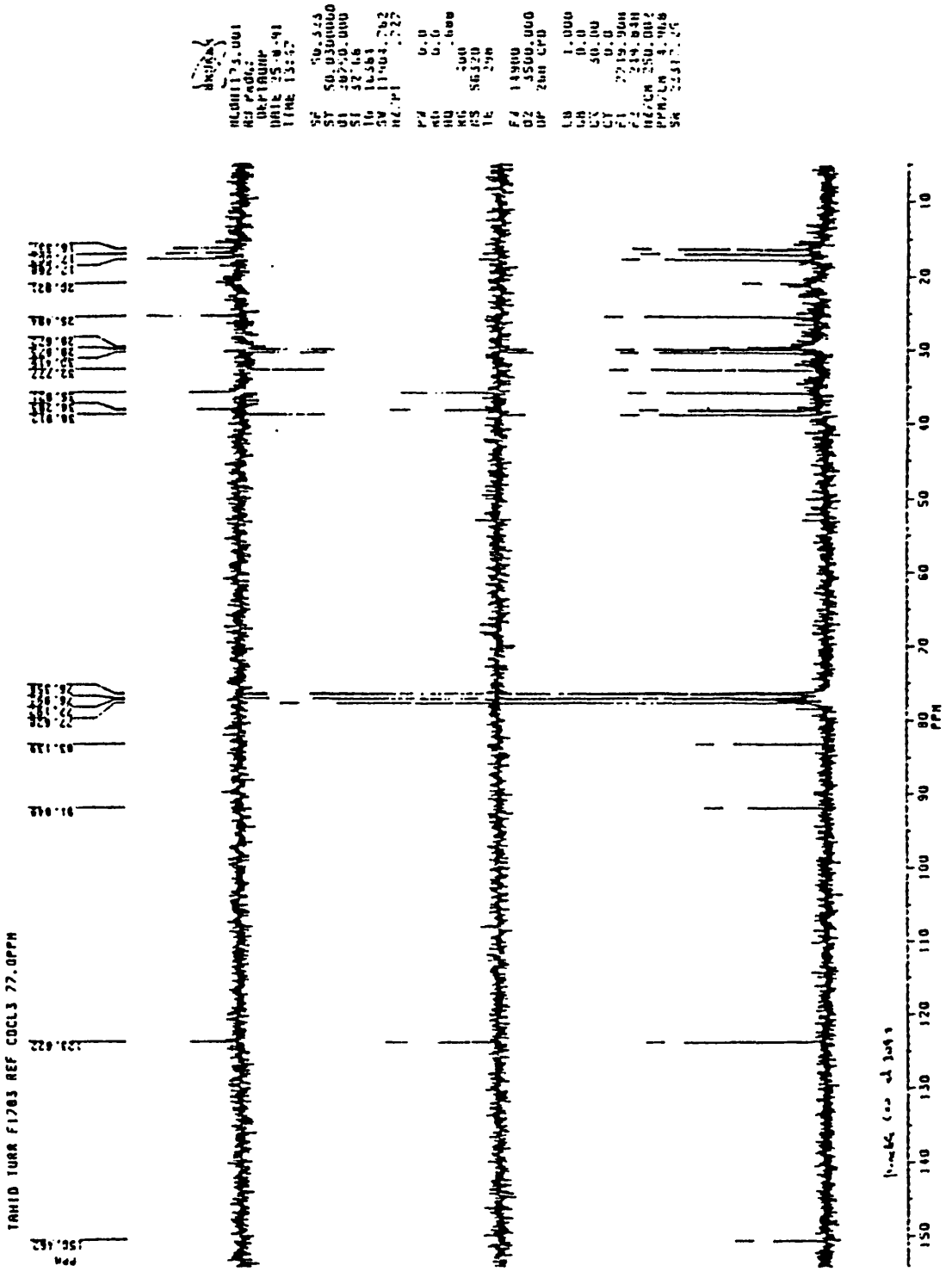


Figure 16. ^{13}C NMR spectrum of F1783 in $CDCl_3$.

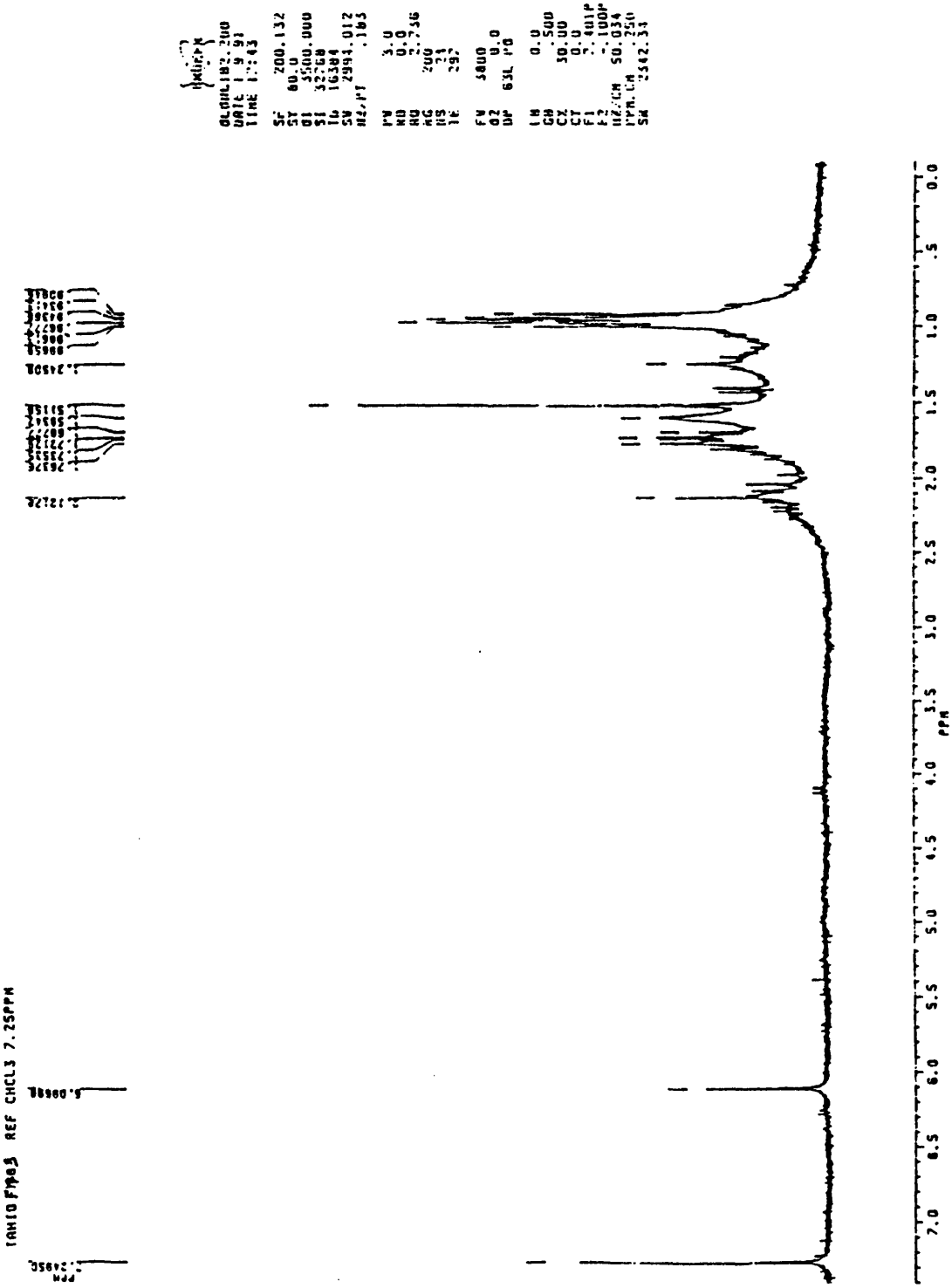


Figure 17. ^{13}C NMR spectrum of F1783 in $CDCl_3$.

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CHAPTER IV

TOONA AUSTRALIS

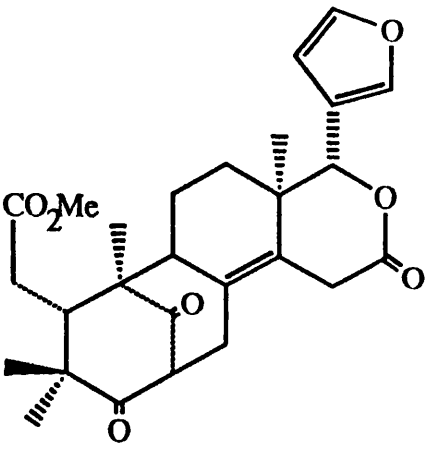
INTRODUCTION

The only genera of the Cedrela tribe, subfamily Swietenioideae, are *Cedrela* and *Toona*. The woods of *Cedrela* and *Toona* are difficult to distinguish. These genera are the only two to have been distinguished by chromatography. Chemically *Cedrela* gives mainly mexicanolide (**1**), whereas *Toona* does not[1]. The wood of *Cedrela* has a faintly aromatic odour mainly due to the presence of a golden yellow essential oil. In 1931 Mell reported that the wood was an interesting source of a natural dyestuff[2].

Toona ciliata , commonly knows as Tun in India, is a tall handsome tree, about 50 - 60 feet high. It is found in abundance in the sub Himalayan region and in other Asian countries such as Burma, Malaysia and Indonesia.[2,3] Earlier investigations of the timber of *Toona ciliata* Roxb have resulted in the isolation of cedrelone (**2**)[4,5] and a number of sesquiterpenoids.[5] Chatterjee and his colleagues have isolated the chemical constituents of two varieties of *Toona ciliata* Roxb. which are available in West Bengal. One variety produces cedrelone (**2**), 1,2-dihydrocedrelone (**3**), bergapten (**4**), and β-sitosterol (**5**), and the other produces two tetranortriterpenoids and a coumarin. The occurrence of 1,2-dihydrocedrelone provides further interesting evidence of the chemotaxonomic features of the Meliaceae.[6]

Two novel ring B cleaved tetranortriterpenoids with antifeeding activity, toonacilin (**6**) and 6-acetoxy toonacilin (**7**), have been isolated from leaves of *Toona ciliata* M. J. Roem var australis[7]. The two compounds are the first B-*seco*tetranortriterpenoids, with an intact ring D, which are related to cedrelone (**2**).

Toonafolin (**8**), the first tetranortriterpenoid ring B lactone found in Meliaceae, has recently been isolated from the ether extract of the leaves of *Toona ciliata*.[8] Two more novel B-*seco*tetranortriterpenoids, 23-(*R*,*S*)-hydroxytoonacilide (**9**) and 21-(*R*, *S*)-hydroxytoonacilide (**10**) have also



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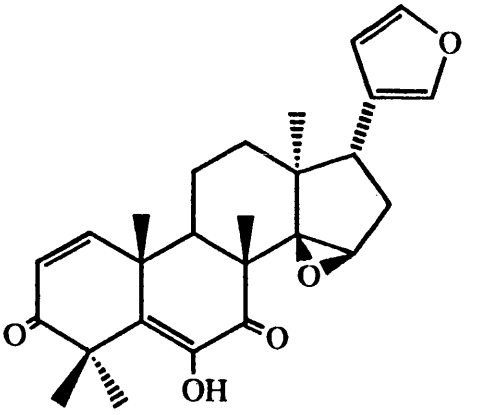

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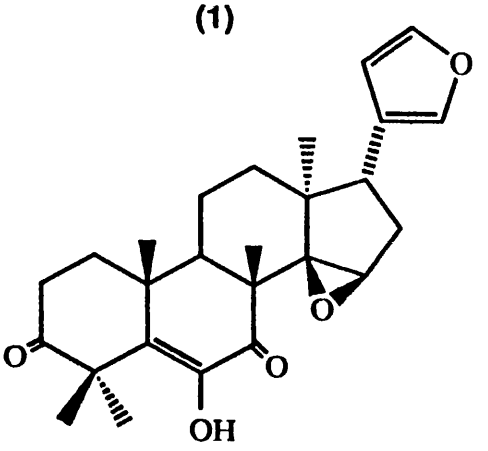

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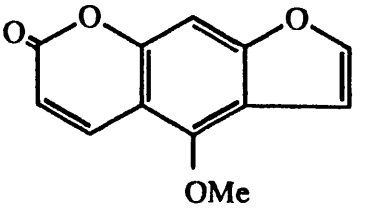

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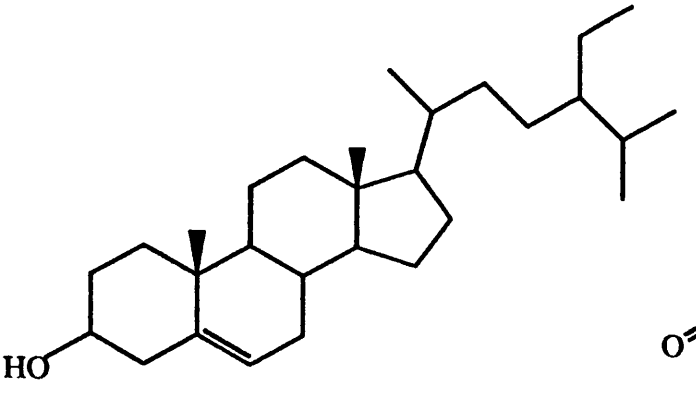

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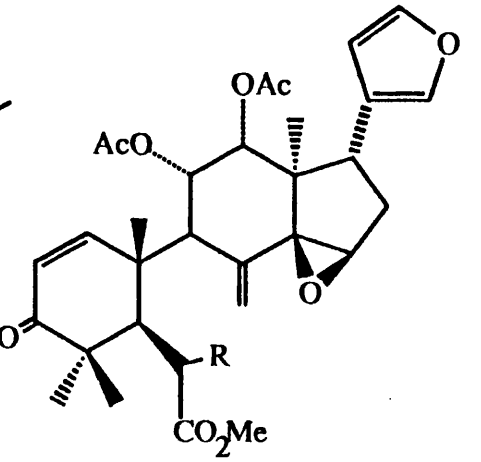
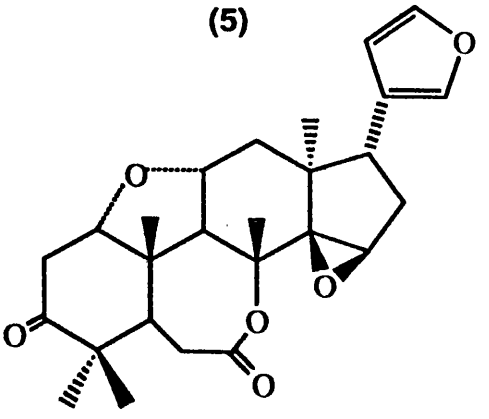


(6) R = H

(7) R = OAc



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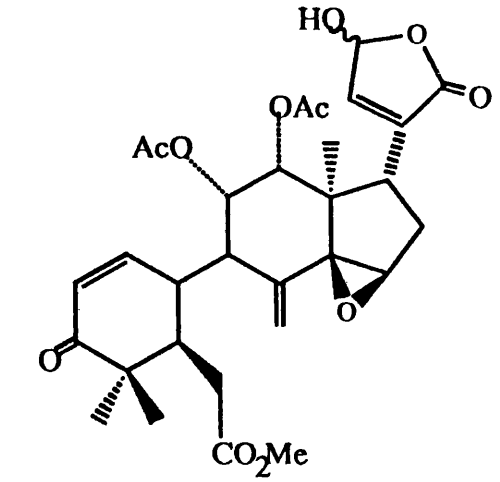

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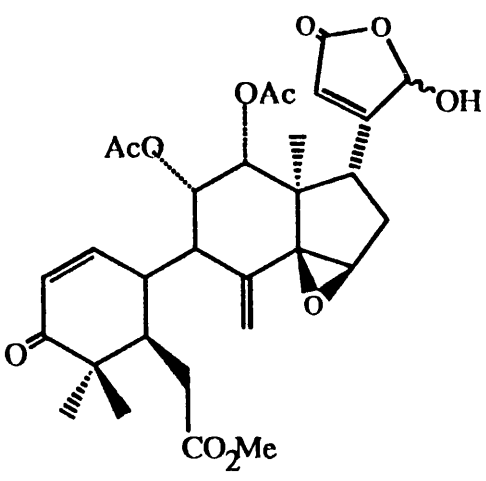

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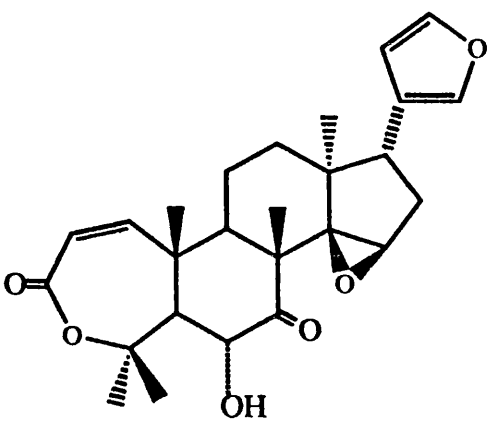

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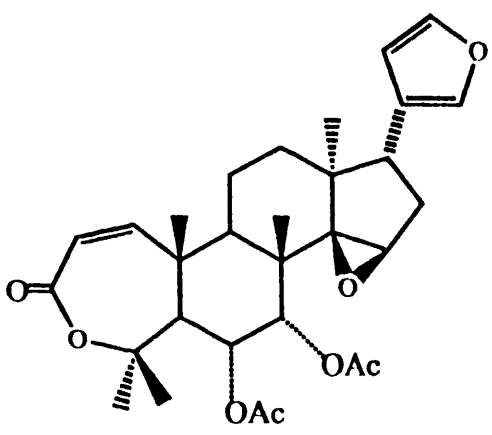

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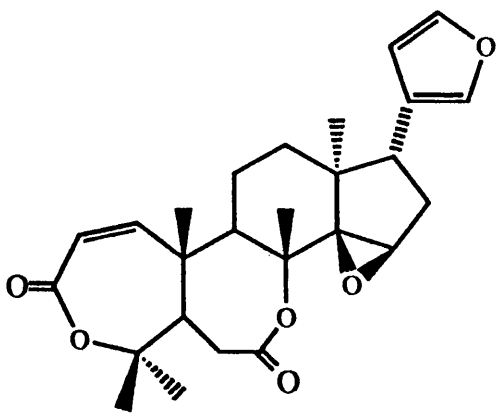

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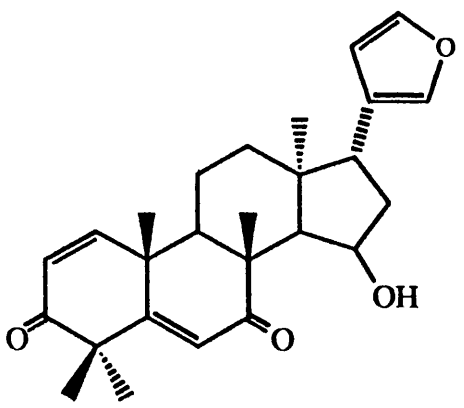


(14)

been isolated from the bark of this plant[8,9].

In 1981 Kraus and Kypke isolated two new tetranortriterpenoids surenone (**11**) and surenin (**12**) from the leaves of *Toona sureni* [Blume] Merrill, collected from Bulolo, Papua New Guinea. The structures of these related compounds were assigned on the basis of their spectroscopic properties[10]. Subsequently, Kraus and his colleagues found surenolactone (**13**), the first tetranortriterpenoid A/B dilactone, from the same plant. Compound (**13**) was isolated from the ether extract of the leaves and its structure was determined from its spectroscopic properties and some chemical transformations.[11]

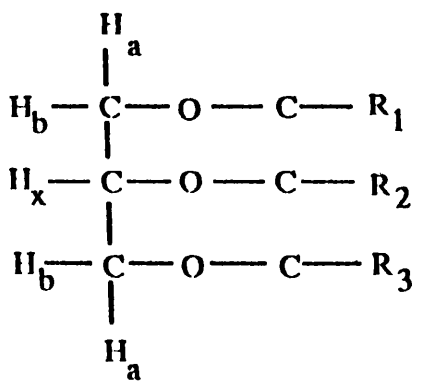
6-Desoxycedrelone (**14**) was isolated by Banerji and Mitra from the benzene extract of *Toona ciliata* heartwood where it occurred with cedrelone. The lack of the diosphenol group is apparently from the relevant spectral data and the negative ferric chloride test. Strictly speaking this compound is a dihydro-6-desoxycedrelone.

In this section we report the results of an investigation of the triterpenoid constituents of a sample of *Toona australis*. The ethyl acetate extract yielded the crystalline tetranortriterpenoid cedrelone (**2**). Small amounts of other tetranortriterpenoids, unfortunately as mixtures, were also isolated together with glycerides and sterols. A large amount of a red solid was obtained. This was very polar and probably arose by a polymerisation reaction on the column. A recent publication by Taylor reports the identification of cedrelone as the main constituent of *Toona australis*.[16]

DISCUSSION

The cut wood sample of the species *Toona australis*, collected in Australia, was kindly supplied by Prof. D. A. H. Taylor. The ethyl acetate extract of the ground bark was chromatographed on a silica gel flash column. Seven different products, labelled A-G, were obtained and were subjected to spectroscopic analysis as discussed below. Some products proved to be mixtures but the small quantities prevented further efforts at separation.

The ^1H NMR spectrum of compound A indicated that it is a glyceride, probably a mixture of similar compounds. A clearly resolved AB(X) multiplet pattern at δ_H 4.15 is typical of glycerides and is due to the coupling of two non-equivalent nuclei H_A and H_B at each end (1 and 3) of the glycerol structure. This leads to a pair of doublets J_{AB} = 11 Hz. These signals are split further into secondary pairs of doublets by the coupling of the hydrogen attached to H_X at C_2. This hydrogen appears further downfield.

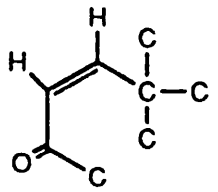


There are signals at between δ_H 5.0 and δ_H 5.5 due to vinyl hydrogens suggesting unsaturation in the fatty acid chains. The polymethylene chains appear at δ_H 1.2 with the terminal methyl group triplets at δ_H 0.9.

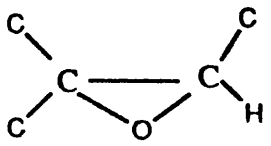
Compound B is a mixture of sterols. Its ^1H spectrum is difficult to interpret due to the complexity of signals between δ_H 0.0 and δ 3.0. However it is

possible to pick out some functionality. Thus there are cyclopropane signals at δ_H 0.3 and 0.6 (both, d), vinyl methyls at δ_H 1.55 and 1.64 and vinyl protons at δ_H 5.10 (m). It was not possible to progress further with this product.

Compound C is cedrelone (**2**), a member of havanensin group of limonoids (all rings A, B, C, and D intact). This is confirmed by its ^1H and ^{13}C NMR spectroscopic data. Analysis of ^{13}C DEPT spectrum indicated a molecular formula $C_{26}H_{30}O_5$ and revealed the presence of 5 quartets, 3 triplets, 8 doublets and 10 singlets. Analysis of the ^1H NMR spectrum confirmed the presence of five tertiary methyl groups, a β-monosubstituted furan ring, an enone chromophore type (a), and an epoxide group as in (b) at δ_H 3.72.



(a)



(b)

The enone group (a) can only be placed in the terminal A ring, the carbonyl group corresponding to the ubiquitous 3-oxygen function. The relative position of the diosphenol group and the epoxide ring can be seen from the NMR data since H-15 is strongly deshielded by the 7-carbonyl group. The experimental values of δ_H and the coupling constants J are in accord with the literature[13] (see Table 1).

An interesting feature of the ^1H spectrum is the apparent lack of coupling between H-15 and the two non equivalent H-16_a and H-16_b. This may be due to ring inversion of ring D so as to alter the dihedral angles sufficiently to show no coupling at room temperature. Thus far the ^{13}C NMR data of cedrelone have not been found in literature. The assignment of the chemical shifts based on the ^{13}C DEPT spectrum are given in Table 2.

Compound D is β-sitosterol (5), the ubiquitous sterol of higher plants. β-Sitosterol was first reported from *Toona ciliata* heartwood by Banerji in 1975[12]. It was readily identified on TLC by comparison with an authentic sample of β-sitosterol.

Compound E is a mixture of three different compounds (E1-3). Closer inspection of the ^1H NMR spectrum shows three different intensities. It is possible that all three of these compounds are limonoids as the ^1H spectrum shows functionality typical of this group. All three compounds contain a ring A enone. These are characterised by the three pairs of doublets found between δ_H 6.0 and δ_H 7.5 (δ_H 6.10 and 6.95 ; 6.75 and 7.20 ; 6.75 and 7.35). E1 has furan hydrogens, one of which can be clearly seen at δ_H 6.5, but the others do not. This is presumably because the furan has been oxidised in these two compounds. This is fairly common, e.g. gedunin converting to photogedunin.[15] It was difficult to assign structures to the components of this mixture.

Compound F, a pure component, is probably limonoid since it shows typical functionality in both the ^{13}C and ^1H spectra. The functionality is based on the ^1H spectral data as follow: four methyl groups at δ_H 1.30, 1.28, 1.52 and 1.61, a ring A enone at δ_H 5.98, and 7.10 and two furan hydrogens at δ_H 6.5 and 7.50. There is obviously no epoxide hydrogen through there is a broadish singlet at δ_H 3.0. This could mean that ring D has rearranged from a 14, 15-epoxide to a 15-ketone function. There are also two hydrogens coupling together at δ_H 4.60 (m) and δ_H 5.65 (d, J=14 Hz). These could be vicinal hydrogens at position 6 and7 or 11 and 12. Both positions appear to be acetylated.

The ^{13}C spectrum for compound F was very weak due to low sample concentration. The following signals could be observed as enone carbons C-1 and C-2 at δ_C 156.4 and 126.5, furan carbons (quaternary not observed) at δ_C 142.9 140.3 and 109.6, methine carbons probably C-6 and C-7 or

C-11 and C-12, and methyl carbons at δ_C 14.0 - 25.0. There is still a lack of information for the assignment of the structure of compound F. It is not obvious what has happened to ring D or, indeed, why all the proton methyl signals are deshielded.

Compound G is a deep red solid. It was the largest component of the ethyl acetate extract. It is probable that this compound is a lignan polymer since the NMR spectra show extensive aromatic and vinylic protons (δ_H 5.8 - 7.1) which are typical of these compounds. Due to the extreme complexity of the multiplets it hard to conclude anything from the spectra. The ^{13}C spectrum showed much signal broadening because of *inter* or *intra*molecular crosslinking.

EXPERIMENTAL

The dried ground wood(448.9 g) was extracted using a soxhlet extractor, first with ethyl acetate and then with methanol. Each extraction took about 20 hours for the sample to be completely extracted into 2.5 litres of the solvent. Evaporation of the ethyl acetate *in vacuo* gave a dark brown residue (17 g). The dried extract was redissolved in small amount of ether and subjected to crude separation on dry column chromatography using silica gel (100 g) as the stationary phase. The column was first eluted with petroleum ether followed by increasing proportions of ethyl acetate in petroleum ether and then with increasing proportions of methanol in ethyl acetate. A total of 56 fractions (50 ml each) were collected from the column.

For evaluation and grouping, fractions were analysed on silica gel GF_{254} TLC plates using a mixture of petroleum ether, ethyl acetate and methanol as the developing solvent. Fractions 1 to 18 were run twice using petroleum ether-ethyl acetate (85-15), fractions 19 to 28 with petroleum ether-ethyl acetate (50-50) and fractions 29 to 36 with ethyl acetate-methanol (99-1). Fractions 37 to 56 were very polar and were discarded. For identification of spots, each plate was examined under a UV lamp (254 and 366 nm) and then sprayed with 25% sulfuric acid followed by heating.

Fraction 8 (760 mg) which contained the same spots as fractions 1 to 14, was further fractionated on a flash column of silica gel and eluted with increasing proportions of ethyl acetate in petroleum ether. A total of 18 fractions (labelled 8A-8R) of 20 ml each was collected from the column. The fractions were chromatographed on silica gel analytical plates eluting twice with petroleum ether-ethyl acetate (85-15).

Fraction 8C was evaporated to yield the glyceride (compound A) as a yellow oil with a fragrant smell. Fractions 8G, 8H and 8I, which fluoresced strongly in the UV (366 nm), were combined and rerun on silica gel

preparative plates. The plates were developed in petroleum ether-ethyl acetate (85-15). The main band afforded compound B as a mixture of sterols.

Qualitative TLC analysis of fractions 9-14 indicated that there were 2 major spots close together, after spraying with 25% sulfuric acid. The lower spot was β-sitosterol (compound D), identified by comparison with a β-sitosterol standard. The upper spot afforded more interesting results. Fractions 12, 13 and 14 were combined for separation and identification of the upper compound. Evaporation of these combined fractions afforded nice crystals which were found to be quite insoluble in ether. The crystals (compound C) were recrystallised from ethyl acetate and had m.p. 211-215°C. Compound C was identified as cedrelone (2) m.p. 209-214°C.[13]

Fractions 16, 17 and 18 were combined and chromatographed on four silica gel GF_{254} preparative plates. These plates were developed three times in ethyl acetate-petroleum ether (20-80). Two of the five band were removed and purified by further preparative TLC. The separation yielded 23 mg of compound E (23 mg), a mixture of limonoids, and compound F (17 mg) probably another limonoid. The limited amount of material available prevented further progress with these minor constituents.

Fraction 29 contained the deep red solid (compound G) which was the major component of the extract. Compound G appeared to be polymeric in nature.

Table 1. Comparison data of the experimental chemical shift values of cedrelone with the literature[12].

Proton	Experimental (200MHz) (ppm)	Literature (60 M Hz) (ppm)
H - 1	6.85	6.90
H - 2	6.05	6.10
H - 15	3.74	3.78
4a - Me	1.06	1.12
4b - Me	1.23	1.29
8 - Me	1.43	1.50
10 - Me	1.51	1.56
13 - Me	0.69	0.75
Furans	6.12	6.17
	7.08	7.14
	7.31	7.36

Signal at 6.49 ; -OH (C-6).

Table 2. ^{13}C Shift Assignments of cedrelone.

Chemical shifts (ppm)	Assignments
197.7 , 203.5	-α, β-Unsaturated ketones (C-3 & C-7).
127.1 , 153.3	-Enone carbons (C-1 & C-2).
110.5, 139.2, 123.0, 110.5	-Furan carbons (C-20, C-21, C-22 & C-23).
141.0	-Enol carbon (C-6).
133.6	-Substituted vinylic carbon (C-5).
54.9 , 69.6	-Epoxide carbons (C-14 & C-15).
19.3, 31.7, 34.9	-Methylene carbons (C-11, C-12 & C-16).
20.1,21.0, 22.9, 23.7, 26.6	-Methyls (C-18, C-19, C-28, C-29 & C-26).

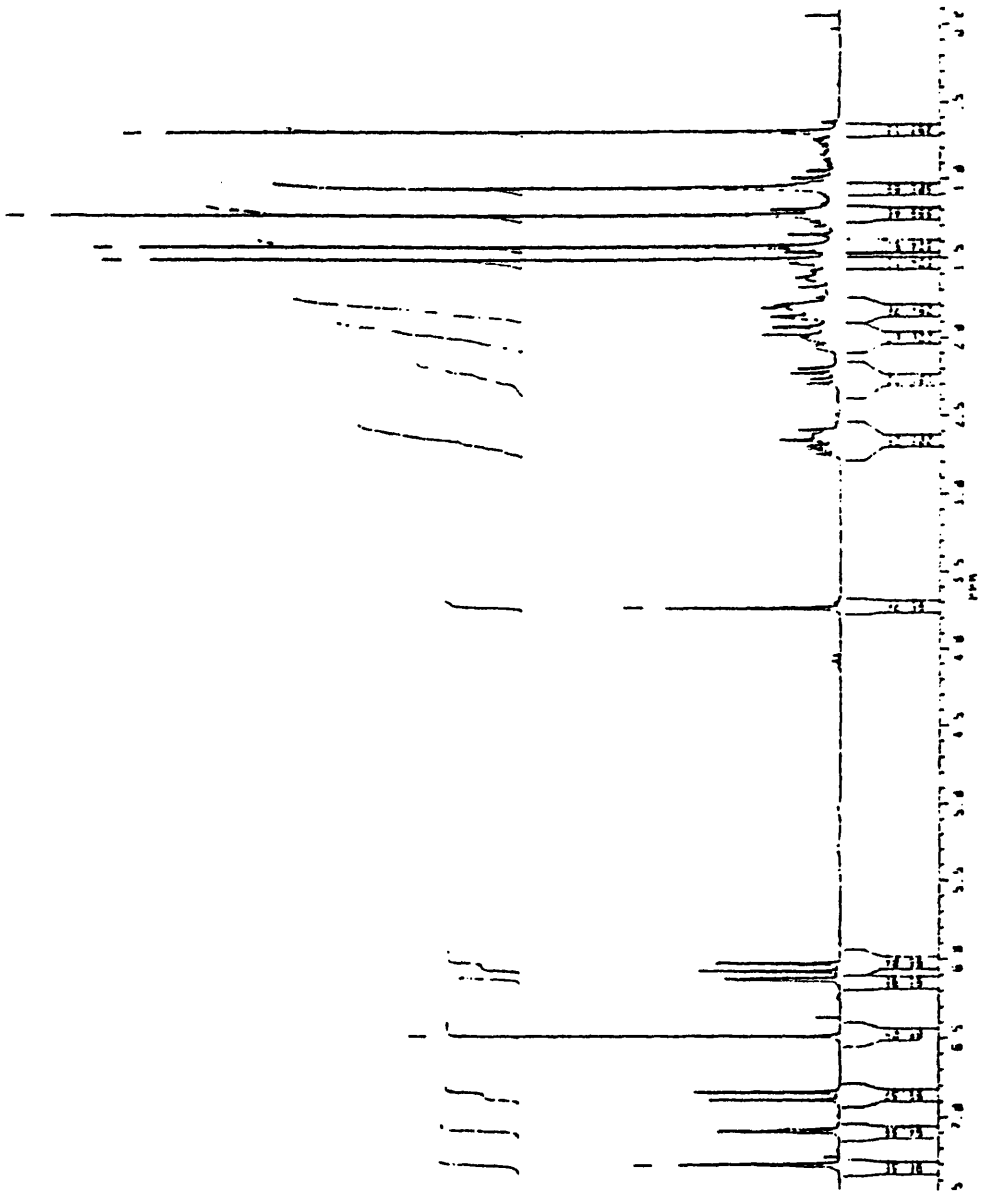


Figure 1. ^1H NMR spectrum of cedrelone (comp. C) in $CDCl_3$.

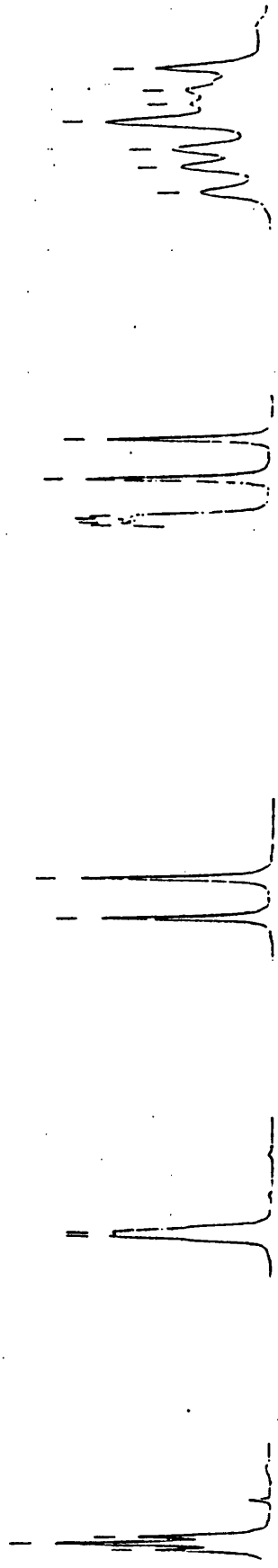


Figure 1.a. Expansion of ^1H NMR spectrum of cedrelone in $CDCl_3$.

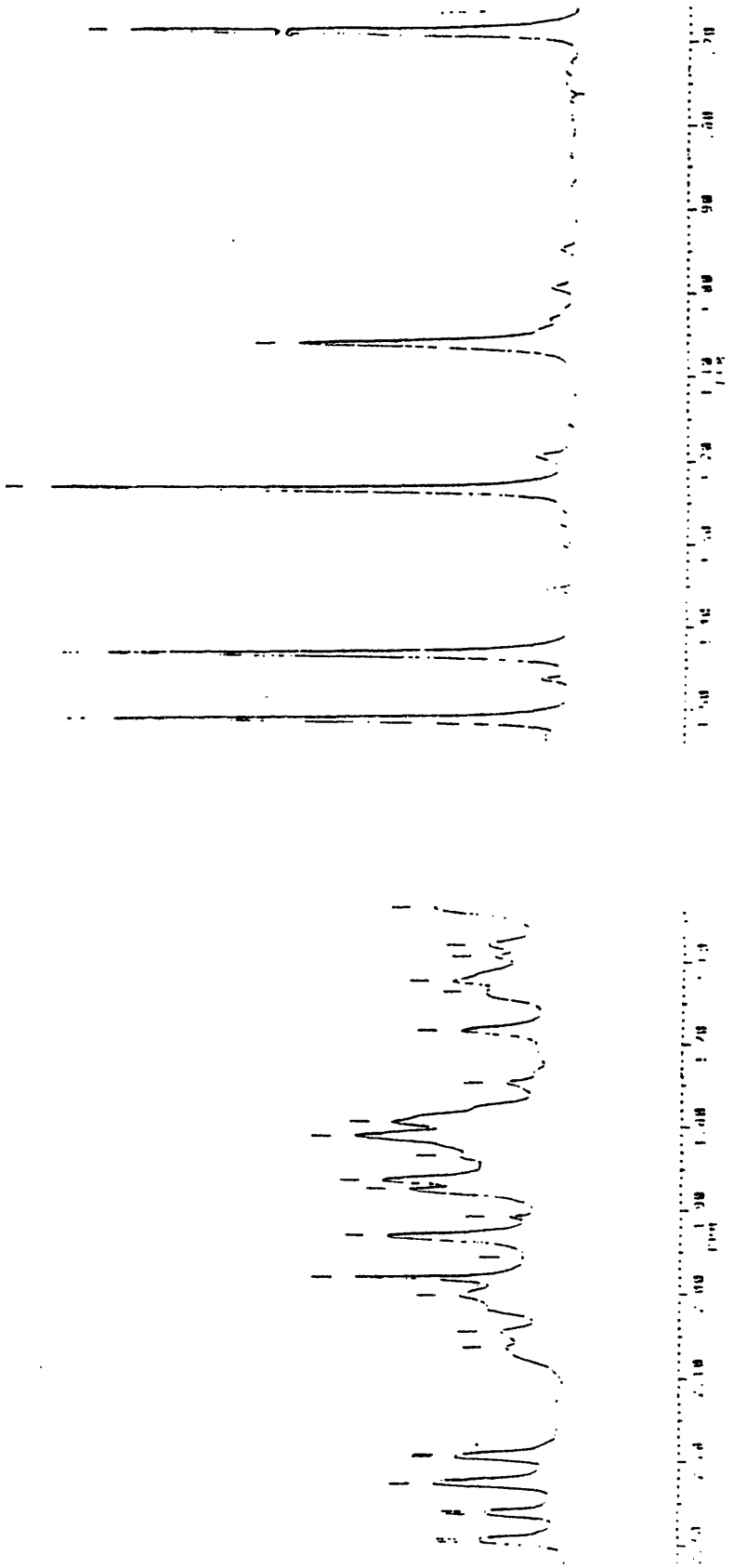


Figure 1.b. Expansion of ^1H NMR spectrum of cedrelone in $CDCl_3$.

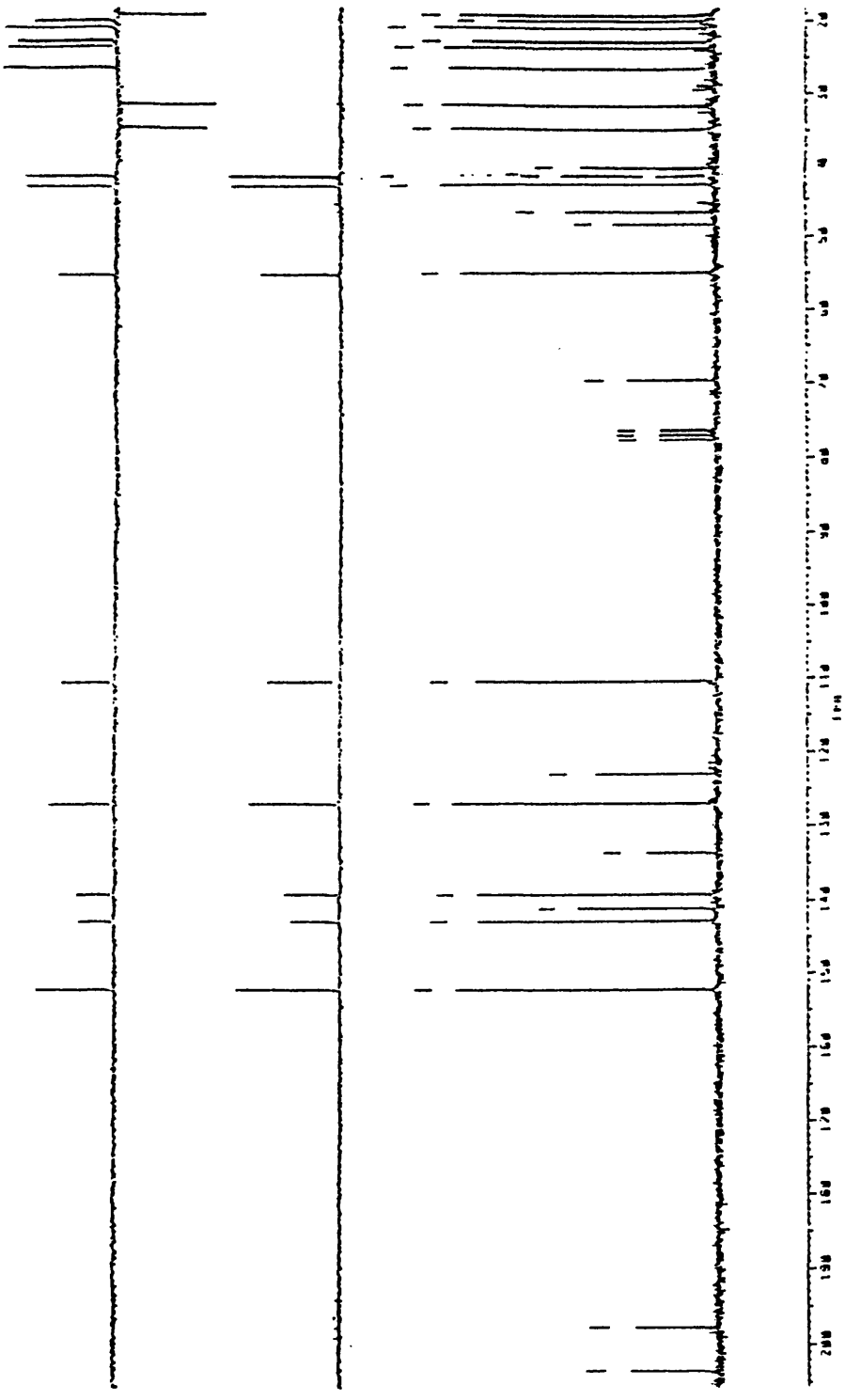


Figure 2. ^{13}C NMR spectrum of cedrelone (comp. C) in $CDCl_3$.

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CHAPTER V

HEMIGRAPHIS ALTERNATA

INTRODUCTION

Hemigraphis alternata originally comes from the east part of Indonesia.[1] Now this plant can easily be found in Java when it is called "kecibeling". It is also cultivated elsewhere. Boorsma[2] indicated that the leaves may be diuretic on account of the high potassium content. According to the natives, the leaves have styptic properties and they are used to treat dysentery, hemorrhage after parturition and hemorrhoids. This plant is also used as a traditonal medicine for dissolving kidney stones. Research on the identification of the active compounds of this plant has not yet been reported. In this section we report an investigation of the leaves of *H. alternata*. From the petroleum ether extract a mixture of two closely related compounds, possibly diterpenoids, was isolated. The spectroscopic data of this mixture is presented. Unfortunately it did not prove possible to assign any structure.

DISCUSSION

The leaves of *Hemigraphis alternata* were collected from Ciamis, West-Java in Indonesia. Preparative TLC separation of combined fractions G5 from the flash column chromatography of the petroleum ether extract of the leaves yielded b-sitosterol and compound G5B1 which appeared to be of some interest. Its spectroscopic properties suggested it was a mixture. Further preparative TLC failed to result in any separation of the mixture.

From the ^{1}H and ^{13}C NMR spectra (Figure 1 and 2) it is possible to deduce that there are two components in the mixture in a ratio of 2 : 1. Each component contains two benzoates, two acetates and a vinyl group ($-CH=CH_2$). Several ketonic carbonyls are present but it is not clear how they are distributed. A similar situation pertains for the oxygenated methines (at least four in each component) and the methines, methylenes and methyls. Two or three singlet carbons are present in each. It is difficult to count carbons with any certainty. Molecular formulae could be from $C_{36}H_{40}O_{12}$ to $C_{37}H_{38}O_{12}$. Its IR spectrum has bands (benzoates and acetates) at 1730 cm^{-1} and 1760 cm^{-1}, (hydroxyl groups) at 3580 cm^{-1} and 3625 cm^{-1}, and 2860 - 3040cm^{-1} (aromatic). The highest peak in the mass spectra is m/z 647 which is presumably not the molecular ion. Subtraction of the carbons of the benzoates and the acetates leaves a C_{18} compound. This suggest the possibility of a diterpenoid but it is not easy to decide which skeletal type.

A COSY spectra of G5B1 is shown in Fig. 3. The connectivity is fairly straightforward though as above overlap of the two spectra poses problems in interpretation. We have not succeded in suggesting a structure for these compounds. The solution must await the arrival of a new supply of plant material.

EXPERIMENTAL

The air dried, ground leaves of *H. alternata* (99.55 g) were extracted in turn with hot petroleum ether, ethyl acetate and methanol. Evaporation of each solvent *in vacuo* left a dark green residue (3.38 g) from the petroleum ether, a dark green oily residue (3.55 g) from the ethyl acetate, and a dark brown residue (7.85 g) from the methanol. The dried petroleum ether extract was then treated with small amount of ether and placed on a small column containing silica gel (30 g) for chromatographic separation. The column was eluted with increasing proportions of ethyl acetate in petroleum ether. The eluate was collected in 15 ml fractions and the similar fractions were combined after TLC evaluation. A total 12 combined fractions labelled G1-G12 were obtained.

Separation of G5 on silica gel GF_{254} TLC preparative plates using petroleum ether-ethyl acetate (70-30) as the solvent afforded compound G5B1 and β-sitosterol (G5B2). From the NMR analysis of G5B1 showed that this compound was still in mixture. Replating of G5B1 on silica gel afforded G5B1 as a gum (39.4 mg) which is strongly UV active. The spectroscopic properties are presented in the text.

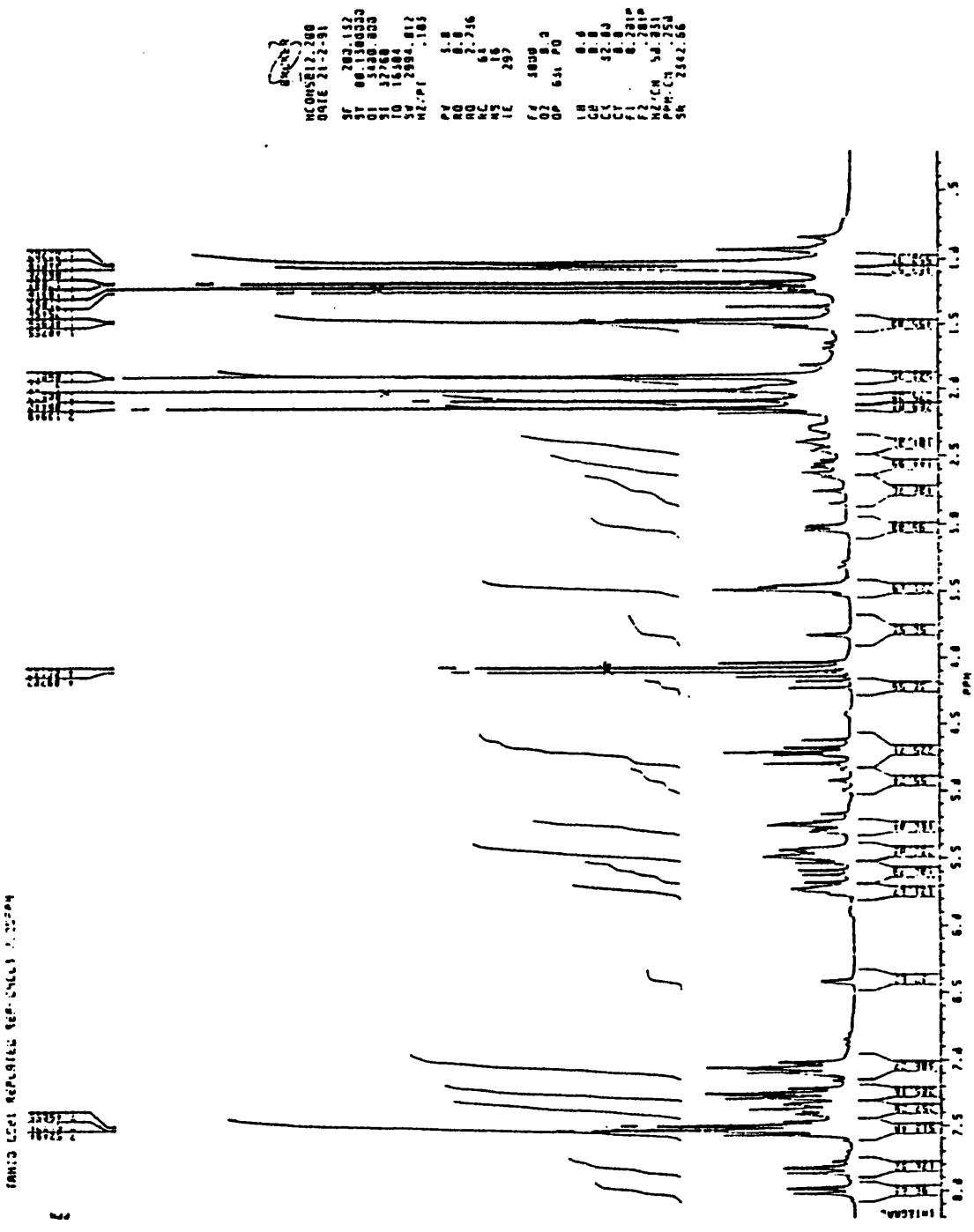

Figure 1. ^1H NMR spectrum of compound G5B1 in $CDCl_3$.

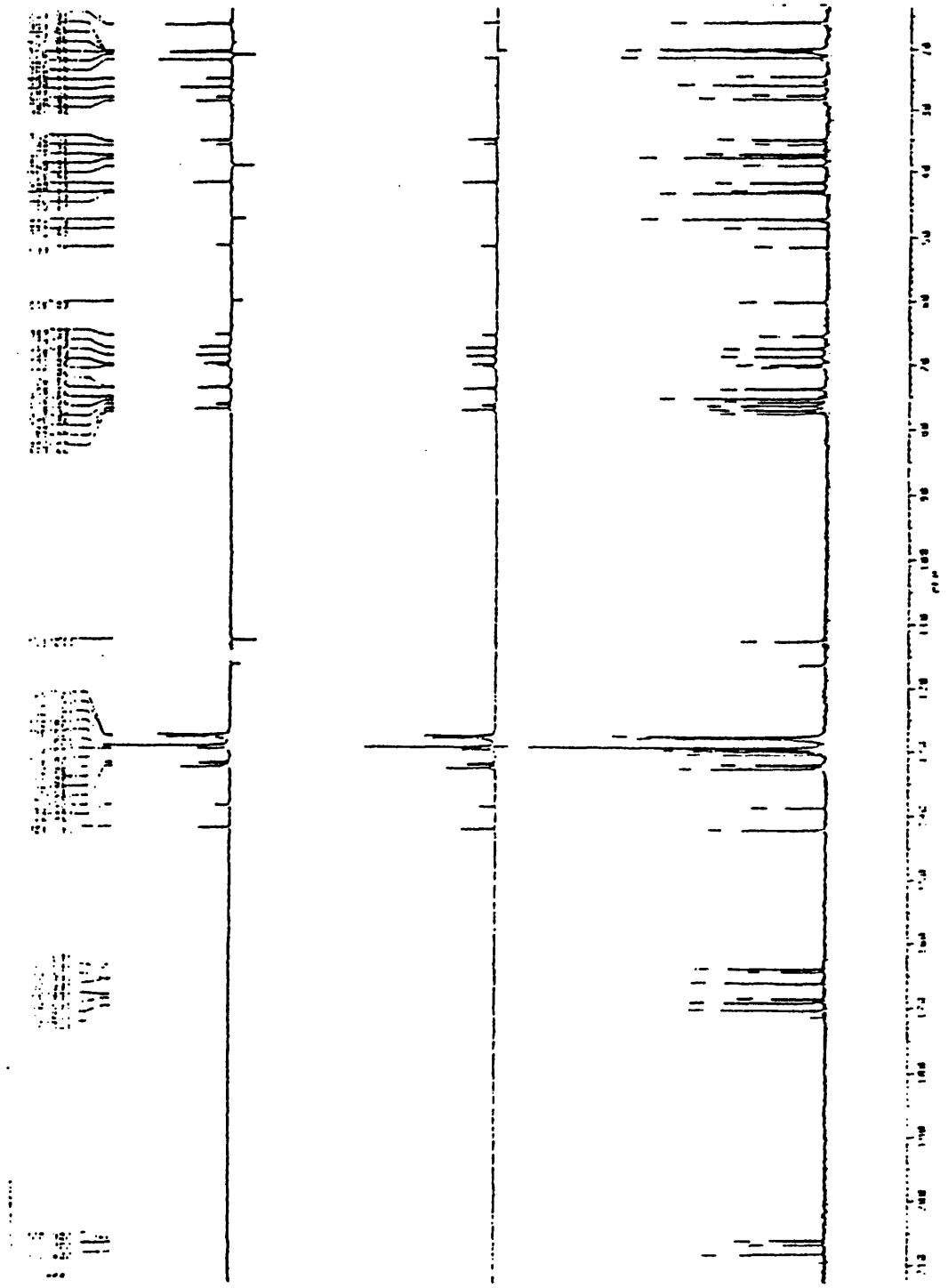

Figure 2. ^{13}C NMR spectrum of compound G581 in $CDCl_3$.

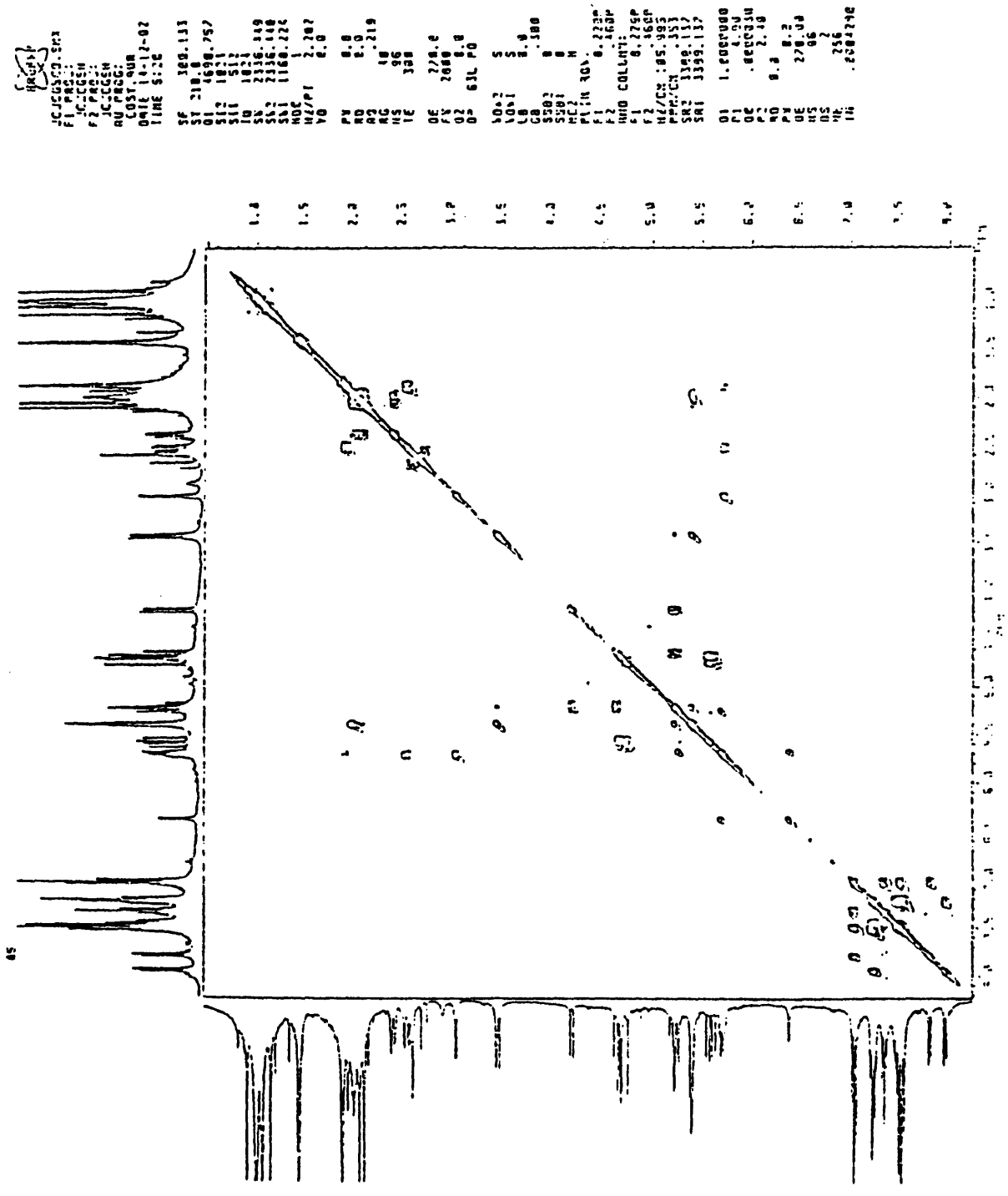

Figure 3. Contour plot of ^1H-^1H COSY spectrum of G5B1 in $CDCl_3$.

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